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JOINT ABNORMALITY AND LEG WEAKNESS IN SWINE

by

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I would like to dedicate this thesis to Mr. Akio Kimura and to the memory of the late Mrs. Midori Kimura. Many thanks for your thoughtful encouragement during my nine years of post-graduate study.

ABSTRACT

Cartilage growth and composition were determined in growing finishing swine (1.6 to 131.0 kg). The effects of age, sex, feed intake and dietary estrogen addition on the incidence of joint lesions were studied. The effects of exercise on the degree of recovery from leg weakness was also examined.

Growth dependent changes in articular and epiphyseal cartilage were observed using 25 boars ranging in age from 3 days to 30 weeks (Chapters 1 and 2). In general, cartilage thickness, cellularity and vascularity decreased ($P < 0.05$) with age. Animal growth was also associated with changes in fine structure and chemical composition of cartilage. The amount of endoplasmic reticulum in the chondrocyte decreased with a concomitant decrease ($P < 0.05$) in the concentration of matrix proteoglycans. Dry matter and collagen concentration increased ($P < 0.05$), and extractability of proteoglycans and the ratio of 4-sulfated to 6-sulfated disaccharide from the chondroitin sulfate fraction decreased ($P < 0.05$) with age.

Chemical composition of the distal femoral articular cartilage from five 20 week old boars was determined in seven different sites (Chapter 3). The concentration of chondroitin sulfate was greater ($P < 0.05$) and that of collagen was less ($P < 0.05$) in the force-bearing than in the non-force-bearing areas of the cartilage.

Joint lesions, examined in 115 pigs ranging in age from 20 to 30 weeks, were observed to be osteochondrotic and to a lesser extent osteoarthrotic (Chapters 1, 2, 4, 5 and 7). Lesion frequency was highest in the elbow and stifle joint, and distal epiphyseal plate of the ulna. Gross, histological and biochemical alterations in abnormal cartilage and bone were determined. Degenerative cartilage showed cell necrosis and loss of matrix proteoglycans. Fibrotic tissue was developed in the area of bone lesions. However, mineralization appeared to be normal in the apparently normal areas adjacent to bone lesions.

Uronic acid in serum and urine were also studied in six 15 week old boars with experimentally induced joint lesions (Chapter 6). It was concluded that measurement of uronic acid in serum or urine is of limited value to detect or verify the presence of degenerative joint lesions.

The incidence and severity of joint lesions increased with age, and were similar among boars, gilts and barrows of a similar age and body weight. Feed restriction and addition of estrogen to the diet were not effective in preventing joint lesions (Chapter 4).

Lame boars allowed an increased exercise area showed no appreciable improvement in locomotory ability (Chapter 7).

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TABLE OF CONTENTS

Page

INTRODUCTION 1

CHAPTER 1 CHANGES IN SWINE KNEE ARTICULAR CARTILAGE DURING GROWTH 3

ABSTRACT 3

INTRODUCTION 4

MATERIALS AND METHODS 5

Experimental Animals 5

Measurement of Cell Density and Histochemistry 6

Chemical Analysis 7

Bacteriological Examinations 9

Statistical Analysis 9

RESULTS 10

Visual Appraisal of Joints 10

Visual Observations of Abnormal Femoral Condyles 10

Cartilage Thickness of Visually Normal Joints 11

Cell Density of Visually Normal Joints . . . 12

Histochemical Observations 12

Biochemical Observations 13

Bacteriological Examinations 15

DISCUSSION 16

REFERENCES 21

Page

CHAPTER 2 AGE RELATED CHANGES AND DEGENERATIVE ABNORMALITIES IN SWINE ARTICULAR AND EPIPHYSEAL CARTILAGE: LIGHT AND ELECTRON MICROSCOPY 36

ABSTRACT 36

INTRODUCTION 37

MATERIALS AND METHODS 38

Animals 38

Thickness Measurements 38

Light Microscopy 38

Electron Miscroscopy 38

Statistical Analysis 39

RESULTS 39

Light Microscopy 39

Electron Microscopy 40

DISCUSSION 42

REFERENCES 45

CHAPTER 3 CHONDROITIN SULFATE DISTRIBUTION IN STIFLE ARTICULAR CARTILAGE OF SWINE 67

ABSTRACT 67

INTRODUCTION 67

MATERIALS AND METHODS 68

RESULTS AND DISCUSSION 69

REFERENCES 72

Page

CHAPTER 4 EFFECTS OF FEED RESTRICTION, SEX AND DIETHYLSTILBESTROL ON THE OCCURRENCE OF JOINT LESIONS WITH SOME HISTOLOGICAL AND BIOCHEMICAL STUDIES OF THE ARTICULAR CARTILAGE OF GROWING-FINISHING SWINE 75

ABSTRACT 75

INTRODUCTION 76

MATERIALS AND METHODS 77

RESULTS AND DISCUSSION 82

Growth Performance 82

Visual Observations 82

Histological and Biochemical Observations . . 86

REFERENCES 90

CHAPTER 5 MINERALIZATION OF NORMAL AND OSTEOCHONDROTIC BONE IN SWINE 108

ABSTRACT 108

MATERIALS AND METHODS 109

RESULTS AND DISCUSSION 111

REFERENCES 115

CHAPTER 6 URONIC ACID LEVELS IN THE SERUM AND URINE OF SWINE WITH EXPERIMENTALLY INDUCED LEG WEAKNESS 123

ABSTRACT 123

MATERIALS AND METHODS 124

RESULTS AND DISCUSSION 125

REFERENCES 128

Page

CHAPTER 7 EFFECT OF HOUSING SYSTEM ON THE RECOVERY OF BOARS FROM LEG WEAKNESS 134

ABSTRACT 134

MATERIALS AND METHODS 135

RESULTS 138

DISCUSSION 142

REFERENCES 145

GENERAL CONCLUSIONS 156

INTRODUCTION

Degenerative joint abnormality and leg weakness, particularly of breeding animals, are a serious problem in the swine industry. In North American and European countries, it has been reported that 10 to 30% of boars in performance test stations are culled because of leg weakness. In spite of its importance, limited research has been conducted on this problem. The etiology of the abnormality is not well understood. Rapid growth rate, lack of exercise and type of floor have been implicated. The major objectives of this study were to investigate the etiological factors of joint lesions and leg weakness in swine.

A series of experiments were designed to study:

1) Normal growth patterns of swine articular and epiphyseal cartilage,
2) Site dependent differences in the chemical composition of cartilage in the stifle joint,
3) Visual, histological and biochemical alterations in degenerative articular and epiphyseal cartilage and bone,
4) Mineralization of normal bones and those with a degenerative condition,
5) Uronic acid in serum and urine from lame pigs,
6) Effects of age, sex, feed intake, growth rate and dietary estrogen on the incidence of joint lesions, and

7) Recovery of boars from leg weakness by changing housing system.

CHAPTER 1*

CHANGES IN SWINE KNEE ARTICULAR CARTILAGE DURING GROWTH

ABSTRACT

Twenty five crossbred boars reared under normal conditions were serially slaughtered at the age of 3 days, 5, 10, 20 and 30 weeks. Five boars were slaughtered at each age and morphological, histochemical and biochemical age related changes in femoral condylar articular cartilage were studied. No osteochondrotic joints were found in pigs 10 weeks of age or younger, while 7 of the 10 boars slaughtered at 20 and 30 weeks of age were osteochondrotic. Cartilage thickness increased ($p < 0.05$) until the age of 5 weeks and decreased ($P < 0.05$) thereafter. Cell density decreased ($P < 0.05$) as age advanced. Age associated changes found in the chemical composition of the cartilage were an increase in the concentration of dry matter and hydroxyproline and a decrease in the concentration of glycosaminoglycans (GAG) including chondroitin sulfate (ChS), keratan sulfate and hyaluronic acid. The proportions of soluble proteoglycan and 4-sulfated disaccharide from the ChS fraction decreased ($P < 0.05$) while the proportion of 6-sulfated disaccharide from ChS increased ($P < 0.05$). Osteochondrosis was observed as a disturbed endochondral ossification, and softening and fracture of the cartilage. The former was accompanied by a loss of intercellular GAG and cell necrosis, and the latter by local losses of GAG and cells. Osteochondrotic cartilage also contained higher proportions of soluble proteoglycan and 6-sulfated disaccharide, and lower proportions of 4-sulfated disaccharide than did the visually normal cartilage.

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INTRODUCTION

Among intensively reared modern swine, degenerative joint diseases often occur, and the incidence and severity of the condition increases with advancing age of animals (Grondalen, 1974a). An understanding of changes that take place in joint cartilage during growth of pigs, therefore, is not only of basic but also of practical importance.

Articular cartilage consists of relatively few cells and abundant extracellular matrix. The cells and the matrix are functionally interdependent. The main role of the cell is to produce and maintain the matrix. In turn, the matrix has an important role to maintain the cells in homeostasis. The matrix contains a large proportion of water, a meshwork of collagen fibers and a non-fibrous amorphous substance (ground substance). The major component of the ground substance is a glycosaminoglycan-protein complex (or proteoglycan), which is responsible for sustaining rigidity of cartilage (Meachim and Stockwell, 1973). Glycosaminoglycan (GAG) depletion is related to softening of articular cartilage (Sokoloff, 1966).

A progressive decline in cell density has been reported in the articular cartilage of human femoral condyles during growth and maturation (Stockwell, 1967). No studies of cell density change with age have been reported for swine articular cartilage. Studies of the overall chemical composition of swine articular cartilage (Simunek and Muir, 1972a) indicated that early postnatal growth is associated with a rapid increase in the concentration of collagen and dry matter, and with a rapid decrease in GAG uronic acid and the proportion of soluble proteoglycan present.

Simunek and Muir (1972b) reported that the proportion of soluble proteoglycan in the cartilage of femoral condyles and patellas was higher in lame than in normal pigs. However there were no apparent differences in dry matter, uronic acid and collagen concentration of the cartilage between lame and normal pigs.

The present study was a morphological, histochemical and biochemical approach with two objectives: 1) to provide more detailed information of the growth pattern of porcine articular cartilage, and 2) to study the composition of the articular cartilage obtained from lame pigs.

MATERIALS AND METHODS

Experimental Animals

Five groups of five newborn litter-mate crossbred (Yorkshire x Lacombe) boars were obtained from the University of Alberta herd. One boar from each of the five litter groups was randomly selected for slaughter at 3 days and 5, 10, 20 and 30 weeks of age. Housing and management of the animals followed standard practices for Alberta as described by Aherne et al. (1974). Pigs were weaned at 5 weeks of age and fed standard starting (from 5 to 10 weeks) and growing (after 10 weeks of age) diets ad libitum. Indoor temperature of the barn was maintained at 22°C. After weaning, pigs were housed as litter groups in concrete floored pens. Because of serial slaughter, the floor area per animal increased from 0.48 m^2 from 5 to 10 weeks, to 3.34 m^2 from 20 to 30 weeks of age. Pigs were slaughtered by mechanical stunning and exsanguination. Immediately after slaughter, all limb joints were opened and visually examined for soundness of articular cartilage. Because of the high incidence and severity of degenerative lesions in the knee (stifle) joint (Grondalen, 1974a), the femoral condyle was selected for morphological, histochemical and biochemical studies. The medial and lateral condyle of each femur were sagitally split in the centre to appraise the soundness of bone. The surface area of each condylar bone was also examined after the removal of cartilage for histochemical and chemical analyses.

The thickness of articular cartilage was measured in the centre of the caudal summit of all apparently normal medial femoral condyles by removing a small piece of cartilage from this area free of subchondral bone. A single measurement was made using vernier calipers.

Measurement of Cell Density and Histochemistry

Three strips of apparently normal articular cartilage and subchondral bone of approximately 2 x 4 mm at the articular surface were taken transversely from the central area of the medial femoral condyle of each left leg. Samples of abnormal cartilage were taken from any location on the femoral condyle of all right and left legs which showed cartilage damage. Corresponding sites of visually normal femoral condylar cartilage were also sampled from the same pig when possible, or from a different pig when it was not possible. All samples were fixed in 10% formalin in 0.1 M phosphate buffer pH7.3. Fixed tissues were routinely decalcified with 20% formic acid, dehydrated, and embedded in paraffin wax (Drury et al., 1967). Seven μ thick sections were cut vertical to the articular surface, and stained with hematoxylin and eosin (Drury et al., 1967), and safranin O, fast green, and iron hematoxylin (Lillie, 1965). Safranin O is an orthochromatic dye which selectively stains GAG (Rosenberg, 1971).

The cell density was estimated in the apparently normal cartilage by counting the number of nuclei using an eye piece micrometer. Ten sections were selected from each strip. Each section was examined in the superficial, middle and deep zones as defined on the basis of morphology and arrangement of the cells by Meachim and Stockwell (1973). Two observations were made within each zone.

Chemical Analysis

Femoral condyles, which had been sealed in plastic bags and stored in a freezer (-30°C), were thawed. Lateral and medial femoral condylar articular cartilage were separated from subchondral bones of both left and right legs of each animal, finely diced and thoroughly mixed. The dry weight of cartilage was determined by acetone drying (Brandt and Muir, 1969). Hydroxyproline was analyzed by the method of Stegemann and Stalder (1967).

To isolate cartilaginous GAG, acetone dried samples (100 to

300 mg) were digested with papain according to Scott (1963). After proteolytic digestion, trichloroacetic acid was added to a final concentration of 10% and the mixture was held at 3°C overnight. The protein precipitate was removed by centrifugation for 15 min at 13000 x g and 0°C. The supernatant was dialyzed for 24 h against each of running tap water and deionized water (Schiller et al., 1961). Cetylpyridinium chloride (CPC) was added to the dialysate to precipitate GAG (Scott, 1963). Following the removal of CPC with potassium thiocyanate saturated ethanol (Lowther et al., 1967), the total GAG obtained was determined as uronic acid (Dische, 1947; Bitter and Muir, 1962) using glucuronolactone as standard. An aliguot of the GAG was then subjected to chondroitinase digestion (Saito et al., 1968). In this process chondroitin sulfate (ChS) was digested with chondroitinase-ABC (Miles Laboratories, Elkhart, Indiana). The digestate was chromatographed on Whatman No. 1 filter paper with standard unsaturated 4-sulfated, 6-sulfated and non-sulfated disaccharide (Miles Laboratories, Elkhart, Indiana) according to the methods of Saito et al. (1968) and Murata and Bjelle (1976). The discrete bands obtained for each ChS were cut from the paper, eluted with distilled water and analyzed for uronic acid to determine the amount of unsaturated disaccharide present. The remaining GAG preparations were combined within each age group, and subjected to hyaluronidase digestion (DiFerrante, 1956) followed by fractionation by means of ion-exchange chromatography as outlined by Schiller et.al. (1961). The susceptibility to hyaluronidase (bovine testicular hyaluronidase, type I, 360 National Formulary Units, Sigma Chemical Co., St. Louis, Missouri)

was measured as percent reduction in turbidity as described by DiFerrante (1956). The GAG were eluted stepwise with 0.5M, 1.5M and 4.0M NaCl from a 0.9 x 44cm column containing 200-400 mesh Dowex 1-X2 in the Cl^- form. Each fraction was assayed for uronic acid and hexose (Trevelyan and Harrison, 1952). Galactose was used as the standard hexose. After fractionation of GAG, each fraction was characterized by electrophoresis on cellulose acetate membranes. Pyridine-formic acid buffer, pH 3.0 (Hata and Nagai, 1972) was used as the electrophoretic medium. Reference GAG standards including ChS, hyaluronic acid (HA), and keratan sulfate (KS) were a gift from Dr. M.B. Mathews, Department of Pediatrics, University of Chicago.

Total and percent 2M $CaCl_2$ soluble proteoglycan were determined as uronic acid by the methods of McDevitt and Muir (1976).

Bacteriological Examinations

Joint swabs were obtained from the stifles of all boars slaughtered at 20 and 30 weeks of age, and cultured for bacteria and mycoplasmas. These examinations were carried out at the Veterinary Services Division, Alberta Department of Agriculture.

Statistical Analysis

Age related changes in cell density and chemical composition were analyzed statistically using analyses of variance. Significant differences among the age means were determined using Newman-Keuls' multiple range test (Steel and Torrie, 1960). The harmonic number of observations per cell was computed ($n_h = 0.395$) and used in the multiple range test.

RESULTS

Visual Appraisal of Joints

No joint defects were found in animals 10 weeks old or younger while all 20 to 30 week old animals showed joint abnormalities in one or more joints. The frequency of lesions was greatest (70%) in the femoral and humeral condyles, but in general the severity of the lesions was greatest in the femoral condyles. The lesions found in the humeral condyles, proximal ulna, tarsi and metatarsi were observed to be slight irregularities or depressions in the articular cartilage. These depressions were independent of the nonarticulating depressions (synovial fossae) which occur normally in some joints during early growth (Sisson, 1917; Doige and Horowitz, 1975).

Visual Observations of Abnormal Femoral Condyles

Four 20 week old animals showed a disturbance of endochondral ossification (osteochondrosis) with small regions of cartilage tissue (1 to 2 mm in diameter and depth) invaginating into the subchondral bone (Table 1). One of these animals demonstrated a superficial fracture of the cartilage, 0.5 x 5.0 mm and extending 1mm below the surface of the cartilage. When pressed with a blunt probe, the lesion area was found to be softer than apparently normal cartilage. Gross morphological changes were not apparent in the subchondral bone tissue of the affected joints.

One of three 30 week old affected animals was not lame, but manifested distrubed endochondral ossification similar to that observed with the 20 week old animals. The other two 30 week old affected pigs were very lame and showed surface fractures and thickening of cartilage. The lesions visible around the fractures ranged from approximately 25 to 200 mm^2 in area and 2 to 7 mm in depth. The lesion areas were partly invaded by membranous tissues that spread from the synovial membrane (pannus formation). Thickened cartilage invaginated (2 to 5 mm) into the subchondral bone, which had more or less collapsed near the osteochondral junction, and contained several small regions of cartilaginous tissue. In longitudinal sections of the femoral condyles, these regions were approximately 1 mm^2 in size and were scattered over approximately 1 cm^2 area adjoining the osteochondral junction.

The term osteochondrosis will be used to describe these abnormalities. The two 30 week old lame boars were considered to be in the advanced stages of osteochondrosis while the non-lame boars were considered to be in the early stages of the disease. The incidence and severity of osteochondrosis were higher in the medial than in the lateral condyle. A visual estimate of the slope of the weight bearing surface from caudal summit to intercondyloid fossa was made for normal and osteochondrotic femoral condyles. The slope appeared to be steeper in normal than in osteochondrotic joints. The steepness of this slope decreased with increasing severity of osteochondrosis (Fig. 1).

Cartilage Thickness of Visually Normal Joints

Cartilage thickness at the centre of the caudal summit of medial femoral condyles increased ($P < 0.05$) during the growth period of 3 days to 5 weeks, and decreased ($P < 0.05$) thereafter (Table 2). A marked decrease in thickness (approximately 3 fold) was observed between 10 and 20 weeks of age. After 20 weeks of age, the thickness remained relatively constant.

Cell Density of Visually Normal Joints

Cell density decreased ($P < 0.05$) from the superficial to the deep zone for each age of animal studied. Within each zone, cell density also decreased ($P < 0.05$) with age (Table 2). The decrease was from 27.6×10^3 to 4.8×10^3 in the superficial, 6.8×10^3 to 0.6×10^3 in the middle, and 2.6×10^3 to 0.4×10^3 cells per mm^2 in the deep zone. However means were not significantly different ($P > 0.05$) between samples taken from the middle zone of 5 and 10 week old boars or between samples taken from the deep zone of 10 and 20 week old boars.

Histochemical Observations

Cartilage cells and matrixes of visually normal joints were stained evenly and intensely with hematoxylin and safranin O. In early osteochondrotic joints from boars aged 20 to 30 weeks, the cartilage without superficial fracture showed similar staining reactions to those of visually normal joints except where there were small areas of ossification failure. Such areas showed cell necrosis and a loss of GAG (Fig. 2). The cartilage tissue with superficial fracture showed a local loss of both GAG and cellularity resulting in tissue fibrillation. There were multinucleated clusters of cells close to the facture (Fig. 3).

In advanced osteochondrotic joints, local loss of GAG was observed in the area of fractured cartilage. In some cases, cartilage near the fracture contained fibrocartilage in the superficial and middle zone. The fibrocartilage extended approximately 25 to 100 mm^2 at the articular surface. A diminished GAG staining reaction was observed in the fibrocartilage. The pannus regions were highly vascular and were also associated with a loss of GAG. The deepest area of invaginating cartilage showed very weak staining reactions of both nuclear material and GAG. Most of the small regions of cartilaginous tissue found in the subchondral bone (Fig. 4) displayed dense cellularity (6163±SD 1106 cells/mm^2) and intense GAG staining reaction. With the exception of the fibrocartilage area, the nonfractured cartilage was histochemically similar to the visually normal cartilage.

Biochemical Observations

Visually normal joints: the chemical composition of visually normal cartilage of the femoral condyles is shown in Table 3. From 3 days to 20 weeks of age, the percentage of dry matter and the hydroxyproline concentration increased ($P < 0.05$), and uronic acid concentration and percent soluble proteoglycan decreased ($P < 0.05$). However from 20 to 30 weeks of age, only hydroxyproline concentration increased significantly ($P < 0.05$). Age related changes in the percent unsaturated disaccharide of ChS were significant ($P < 0.05$) for the 4- and 6-sulfated component. The percent 4-sulfated disaccharide decreased from 68 to 53%, and that of 6-sulfated increased from 20 to 38% during the growth period from 3 days to 30 weeks. However, the percent non-sulfated component was relatively constant at approximately 10% from 3 days to 30 weeks of age.

The cartilaginous GAG were highly susceptible (99.3 to 99.6%) to hyaluronidase in all specimens studied. Each GAG fraction obtained by Dowex 1 column chromatography was determined as uronic acid and hexose. As shown in Table 4, most GAG were eluted with 1.5M NaCl at all ages studied (97.8 to 98.7% of total GAG), while only small amounts were eluted with 0.5M NaCl (1.3 to 2.2%). The uronic acid concentration of the 0.5M and 1.5M NaCl fractions in the dry cartilage decreased with age. The GAG eluted with 4.0M NaCl contained negligible amounts of uronic acid but contained a small amount of hexose, whereas in the total GAG the amount of hexose (as galactose) was less than 1% of that of total uronic acid. The hexose (4.0M NaCl fraction): uronic acid (0.5M + 1.5M NaCl fraction) ratio increased with age.

The GAG fractions were further analyzed by electrophoresis (Fig. 5). Each of the 0.5M, 1.5M and 4.0M NaCl fractions gave a single discrete band with an R_f value similar to that of standard HA, ChS and KS respectively.

Abnormal joints: The concentration of uronic acid, and the proportion of 2M $CaCl_2$ soluble proteoglycan and unsaturated disaccharides of ChS were analyzed. As shown in Table 5, the uronic acid concentration of early osteochondrotic cartilage was similar between tissues without and with superficial fractures. These values were also similar to those of visually normal cartilage from the same age group (Table 3). In the fractured sites of advanced osteochondrotic joints, all tissue samples adjoining those demonstrating weak staining reactions of GAG showed much lower uronic acid concentrations than did similar regions of tissues from visually normal joints (15.0 to 38.4 ± 6.5 mg/g versus 51.3 ± 3.2 to 54.0 ± 2.5 mg/g dry weight). Uronic acid concentrations of cartilage from nonfractured sites in osteochondrotic joints were similar to those from corresponding sites in visually normal joints on a whole thickness basis.

Both the soluble proteoglycan and unsaturated disaccharide of ChS were determined in non-fractured sites of advanced osteochondrotic cartilage and corresponding sites of visually normal and early osteochondrotic cartilage (Table 6). The percent soluble proteoglycan was considerably higher in the advanced osteochondrotic than in the early osteochondrotic and the visually normal tissues. The percent 4-sulfated component of unsaturated disaccharides was lower, and that of 6-sulfated component was higher in the advanced than in the early osteochondrotic and visually normal tissues. The percent non-sulfated component was similar among these tissues.

Bacteriological Examinations

Tests for the presence of pathogenic bacteria and mycoplasmas were negative.

DISCUSSION

The results obtained in this study indicate that the cell density of articular cartilage from femoral condyles decreased rapidly with increasing age of animals (Table 2). This observation is consistent with that of Stockwell (1967) who studied human articular cartilage from femoral condyles and reported that the cell density continuously diminished during maturation (0 to 30 years). There have been no previous reports of changes in the cell density of articular cartilage of pigs. The decline of cell density and the increase of cartilage thickness (Table 2) observed up to the age of 5 weeks suggest enhanced accretion of intercellular matrix per cell during this early growth period.

With advancing age of animals, the percentage of dry matter increased, the uronic acid concentration and the proportion of 2M $CaCl_2$ soluble proteoglycan of the cartilage decreased, and hydroxyproline concentration increased (Table 3). Similar changes have been reported in the cartilage from femoral condyles and patellas of growing pigs (Simunek and Muir, 1972a). The proportion of 4- and 6-sulfated components of ChS also significantly ($P < 0.05$) changed with age, while that of the non-sulfated component did not significantly change. Similar changes have been reported for the 4- and 6-sulfated components of ChS from rabbit costal, cattle nasal (Mathews and Glagov, 1966) and human articular (Greiling and Bauman, 1973 cited by Hall 1976) and costal (Iwata, 1969) cartilages. Murata and Bjelle (1976) have reported the presence of the non-sulfated component in a chondroitinase digested fraction of ChS from pig articular cartilage. However very little information is available regarding the changes in this component with age.

The analysis of GAG revealed the presence of ChS, HA and KS in the articular cartilage of pig femoral condyles (Fig. 5). The age related decrease found in the uronic acid concentrations of 0.5 M and 1.5 M NaCl fractions is associated with decreases in the concentrations of HA and ChS respectively (Table 4). The increasing hexose:uronic acid ratio with age suggests an increase in the proportion of KS in the total GAG (Table 4). Similar changes in ChS and KS of human costal cartilage have been reported (Kaplan and Meyer, 1959), whereas there is very limited information available on the age associated changes in the concentration of cartilaginous HA. The concentrations of KS and HA were very small. Hyaluronidase digestion and ion-exchange chromatographic profiles (Table 4) indicated that the proportion of KS (resistant to hyaluronidase) is less than 1% of the total GAG. The ion-exchange chromatographic profiles also indicated that HA accounted for an average of 1.7% of total uronic acid (Table 4). The presence of HA in pig laryngeal (Hardingham and Muir, 1974), the horse nasal (Szirmai et al., 1967) and whale nasal (Seno and Anno, 1961) cartilage has been reported to be approximately 0.7, 2 to 7 and 2% respectively of total GAG. Although these quantities are small, HA plays an important role as the core polysaccharide to which proteoglycan subunits covalently bind (Hardingham and Muir, 1974). Thus the chain length of HA or its binding ability to proteoglycan subunits affects the molecular size of the proteoglycan aggregate.

Visual observations of abnormal joints indicated that osteochondrosis is mainly manifested as a disturbed endochondral ossification, and softening and fracture of the cartilage. The incidence of osteochondrosis was found to increase with increasing age and liveweight of pigs (Table 1). Similarly Grondalen (1974a) in a study involving Norwegian Landrace pigs ranging in weight from 10 to 80 kg reported that with increasing animal weight the incidence of osteochondrosis increased from 10 to 78%. These observations together with the reduced slope of the weight bearing surface (Fig. 1) observed in the osteochondrotic femoral condyles suggest that the incidence of osteochondrosis is highly related to increasing body weight of pigs. Previous reports have implicated a number of causative agents such as rapid weight gain, conformational abnormality of joints, insecure footing on slippery concrete floors, and muscular weakness due to lack of exercise (Vaughan, 1971; Elliot and Doige, 1973; Grondalen, 1974b). These factors possibly cause a disturbance of cell homeostasis in the cartilage. Histochemically the cartilage resulting in a failure of endochondral ossification showed cell necrosis and a loss of GAG. However, at the present time, no concrete suggestion can be made as to the pathogenesis of the condition.

Histochemical examination in this study indicated that softening of the cartilage was associated with a loss of GAG. This observation is consistent with findings of Sokoloff (1966).

The importance of GAG in the formation of a firm gel of cartilage matrix has been suggested by Kempson et al. (1970) and Meachim and Stockwell (1973).

The cartilaginous tissue found in subchondral bone (Fig. 4) showed both high cellularity and an intense GAG staining reaction, suggesting immature cartilage. However, very little is known of the formation of this tissue. The fibrocartilage observed in the advanced osteochondrotic joints appeared to have been formed for tissue repair as suggested by Howell (1976).

Local depletion of GAG in the fractured area of osteochondrotic cartilage was observed. Similar GAG depletion has been reported in the degenerative articular cartilage of humans (Bollet and Nance, 1966; Byers et al., 1977), and dogs (Lust and Pronsky, 1972). It is generally considered that cartilage degeneration is a focal process (McDevitt, 1973). If the area of GAG depletion is small, it is possible, as was observed in this study, that uronic acid concentrations were similar between cartilage samples collected from visually normal and early (or advanced) osteochondrotic joints when analyzed on a whole femoral condyle basis, or when the cartilage was not separated into superficial and deeper region. This may partially explain the findings of Simunek and Muir (1972b) who studied pig knee joint cartilage by pooling femoral condyles and patellas within a normal or lame group, and reported that there were no differences in the tissue uronic acid concentrations between these groups. In this study it was also found that the pannus region was associated with a loss of GAG. This is consistent with the findings of Otaka and Watanabe (1974).

The proportion of 2M $CaCl_2$ soluble proteoglycan in the cartilage was higher in the advanced osteochondrotic joints than in the early osteochondrotic and visually normal joints. Simunek and Muir (1972b) reported similar differences in solubility between the proteoglycans from lame and normal pigs. These researchers, using gel-chromatography also observed that proteoglycans extracted from the joints of lame pigs were smaller in molecular size. To account for these findings, they suggested participation of lysosomal enzymes which attack proteoglycans of the cartilage matrix.

There is limited information available on the unsaturated disaccharides of ChS of degenerative cartilage. The results obtained in this study indicated that osteochondrotic cartilage contained higher proportions of 6-sulfated and lower proportions of 4-sulfated components. These changes may be the result of: 1) an abnormal process of sulfation on the ChS chain, 2) a decreased synthesis of 4-sulfated disaccharides, or 3) a transfer of sulfate group from the 4 to the 6 position. Further research on ChS biosynthesis in the degenerative cartilage is needed in order to resolve these questions.

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Table 1. Visual appraisal of femoral condyles from serially slaughtered boars

Age	Number of boars	Average body weight (kg)	Number of boars	
			Osteochondrotic	Lame
3 Days	5	1.6	0	0
5 Weeks	5	5.9	0	0
10 Weeks	5	21.0	0	0
20 Weeks	5	80.0	4(2)*	0
30 Weeks	5	131.0	3(2)	2

*Value in parenthesis indicates number of boars which were osteochondrotic in both right and left femoral condyles.

Table 2. Thickness and cell density of visually normal articular cartilage of medial femoral condyles (mean ± SD)

Age	Number of boars	Cartilage thickness(mm)	Cells per mm² Zones Superficial	Middle	Deep
3 Days	5	4.75±0.20a	27555±1816a	6833±330a	2620±183a
5 Weeks	5	7.53±1.10b	16888±1930b	1319± 72b	872± 50b
10 Weeks	5	5.35±1.91a	9313± 121c	1374±120b	594± 43c
20 Weeks	3	1.83±0.38c	5490± 220d	783± 51c	520± 75c
30 Weeks	3	1.59±0.18c	4820± 156e	624± 12d	424± 26d

a-e Means in the same column with differing letters are significantly different ($P<0.05$).

Table 3. Chemical composition of visually normal articular cartilage of femoral condyles (mean ± SD)

Age	Number of boars	Dry matter (% af wet weight)	Hydroxyproline (mg/g dry weight)	Uronic acid (mg/g dry weight)	Soluble Proteoglycan (%)	% Unsaturated disaccharide of ChS		
						4-sulfated	6-sulfated	Non-sulfated
3 Days	5	21.0±0.9a	47.4±4.0a	86.9±02.1a	75.5±2.1a	68±1a	20±3a	12±3a
5 Weeks	5	23.2±0.7b	50.7±2.5a	78.5±1.5b	71.5±2.0b	68±2a	23±2a	9±3a
10 Weeks	5	25.4±0.6c	57.3±1.8b	72.1±1.0c	66.7±2.3c	57±3b	33±4b	10±2a
20 Weeks	3	29.4±0.5d	71.2±0.9c	56.3±1.3d	53.4±1.3d	54±2bc	35±2b	11±2a
30 Weeks	3	30.3±0.5d	72.7±0.5d	55.4±1.9d	51.3±0.7d	53±1c	38±1b	9±1a

a-d Means in the same column with differing letters are significantly different ($P<0.05$).

Table 4. Profile of Dowex 1 column chromatography of articular cartilage GAG from femoral condyles

Age	Uronic acid (mg/g dry tissue) 0.5M NaCl Fraction	Uronic acid (mg/g dry tissue) 1.5M NaCl Fraction	Uronic acid (mg/g dry tissue) 4.0M NaCl Fraction	Hexose (mg/g dry tissue) 4.0M NaCl Fraction	Hexose:uronic acid ratio (x10^{-3})
3 Days	1.9 (2.2)*	83.1 (97.8)	-**	0.3	4
5 Weeks	1.3 (1.7)	75.7 (98.3)	-	0.4	5
10 Weeks	1.1 (1.5)	71.9 (98.5)	-	0.3	4
20 Weeks	0.7 (1.3)	57.3 (98.7)	-	0.4	7
30 Weeks	1.0 (1.8)	56.0 (98.2)	-	0.4	7

* Value in parenthesis indicates % of total uronic acid eluted.
** Negligible amount.

Table 5. Uronic acid concentration of cartilage from osteochondrotic and visually normal femoral condyles

Tissue	Number of tissues analyzed	Uronic acid (mg/g dry weight)
Early osteochondrotic cartilage from 20 week old boars[+]		
Without superficial fracture	3	55.5±2.0[‡]
With superficial fracture	1	54.0
Advanced osteochondrotic cartilage from 30 week old boars[§] [≡]		
Fractured site		
Superficial region	1	18.5
Middle and deep region	3	38.4±6.5
Deepest region of the tissue of abnormal ossification	2	21.1
Fibrocartilage	1	15.0
Non-fractured site	4	52.3±5.5
Visually normal cartilage from 30 week old boars[ϕ]		
Superficial region	3	51.3±3.2
Middle and deep region	3	54.0±2.5
Whole thickness	3	54.3±1.5

[+] Samples were based on whole femoral condyles.

[‡] Standard deviation.

[§] Samples assayed were approximately 3 mg dry weight adjoining those used for histochemical analysis and demonstrating a weak staining reaction for GAG.

[≡] Whole thickness of cartilage was analyzed.

[ϕ] Sampling sites were similar to corresponding sites in advanced osteochondrotic cartilage.

Table 6. Percent 2M $CaCl_2$ soluble proteoglycan and unsaturated disaccharides of ChS in osteochondrotic and normal cartilage

Tissue	Number of tissues analyzed	Soluble proteoglycan (%)	% Unsaturated disaccharide of ChS		
			4-sulfated	6-sulfated	Non-sulfated
Osteochondrotic					
Advanced	3	83.3±3.0[+]	38±1	49±3	13±3
Early	3	53.8±2.5	54±1	35±2	11±2
Visually normal	3	50.6±1.2	53±1	38±2	9±1

[+] Standard deviation.

Fig.1. Transverse section of femoral condyles showing different morphology of weight bearing surface. 1:Visually normal bone from a 20 week old boar. 2:Osteochondrotic(early stage) bone from a 20 week boar. 3:Osteochondrotic(advanced stage) bone from a 30 week old boar. f:intercondyloid fossa. s:summit of medial condyle. Arrow indicates the site of a failure of endochondral ossification.

Fig.2. Section vertical to the articular surface from osteochondrotic cartilage. a:Deep layer of cartilage with intense staining reaction of GAG and nuclear material. b:Cartilage resulting from a failure of endochondral ossification. Cells are necrotic and GAG staining reaction is negligible. c:Bone. Section was stained with safranin-O, fast green and iron-hematoxylin. Scale bar = 0.15 mm.

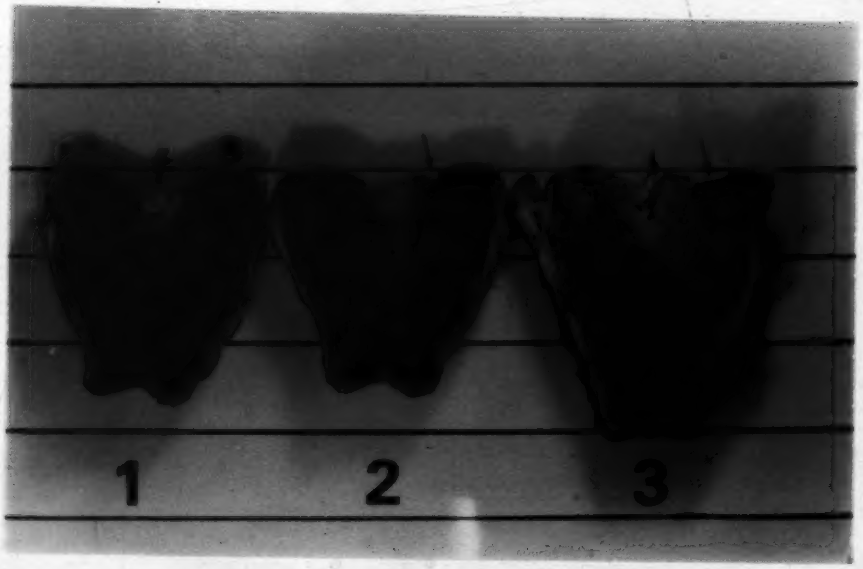
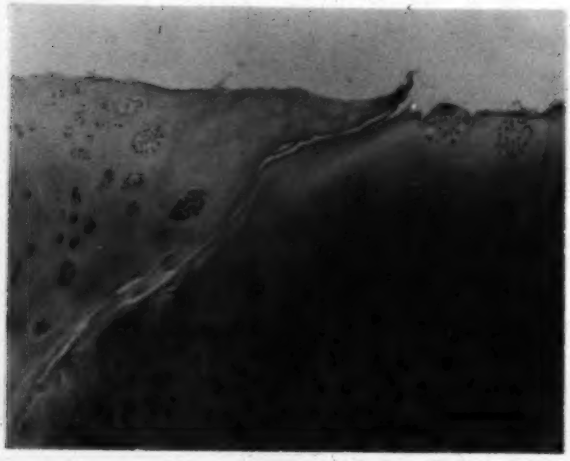

1
2
3

Fig.3. Section vertical to the articular surface from osteochondrotic cartilage with a superficial fracture. The tissue shows weak staining reaction of GAG. Tissue fibrillation, cell clustering and a loss of cellularity are also observed. Section was stained with safranin-O, fast green and iron-hematoxylin.
Scale bar = 0.5 mm.

Fig.4. Trasverse section of medial femoral condyle showing cartilaginous tissue in the subchondral bone. Arrow indicates region of cartilage in the subchondral bone. A: Deep zone of cartilage. B: Subchondral bone. C: Cartilage partially ossified. Section was stained with hematoxylin and eosin.
Scale bar = 0.5 mm.



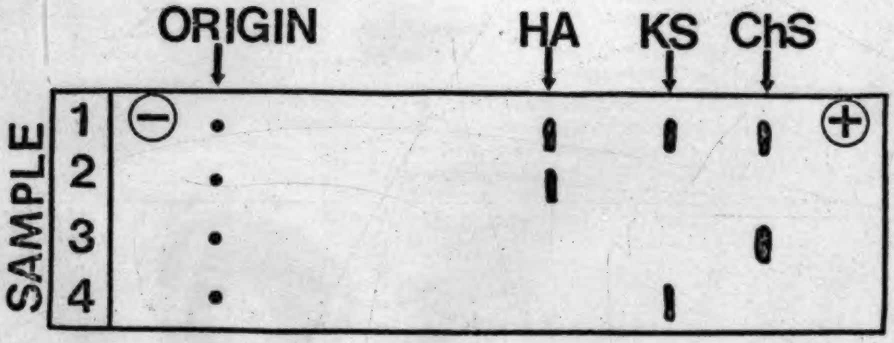

ORIGIN
HA
KS
ChS
SAMPLE
1
2
3
4
⊖
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Fig. 5. Typical electrophoretic pattern of each GAG fraction on cellulose acetate membrane in pyridine-formic acid buffer. Sample: 1, mixture of standard GAG containing HA,KS and ChS; 2, 0.5 M NaCl fraction; 3, 1.5 M NaCl fraction; 4, 4.0 M NaCl fraction.

CHAPTER 2

AGE RELATED CHANGES AND DEGENERATIVE ABNORMALITIES IN SWINE ARTICULAR AND EPIPHYSEAL CARTILAGE: LIGHT AND ELECTRON MICROSCOPY*

ABSTRACT

Age related changes and degenerative lesions were studied using light and electron microscopy in articular and epiphyseal cartilage of boars ranging in age from 3 days to 30 weeks.

Thickness, cellularity and vascularity of both the epiphyseal and articular cartilage, decreased with advancing age. Degenerative lesions were observed as failure of endochondral ossification, and separation and space formation in epiphyseal cartilage. The incidence of cartilage lesions increased with age.

Electron microscopy showed a continuous fibrillar layer (lamina splendens) on the surface of articular cartilage. This layer increased in thickness during growth of animals, and showed accumulation of amorphous material between the fibrils. Collagen fibril diameter increased as age advanced with concomitant randomization of the fibres. Chondrocytes from 3 day and 5 week old animals had a well developed endoplasmic reticulum suggesting very active protein synthesis. While chondrocytes from 10 to 30 week old animals had a reduced amount of endoplasmic reticulum and an increased amount of other organelles such as Golgi apparatus, lysosomes and vesicles.

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INTRODUCTION

During growth, the extracellular matrix and the chondrocytes of cartilage undergo a "maturation process". The underlying biochemical mechanisms leading to maturation are controlled by genetic (Silberberg and Silberberg, 1941; Silberberg et al., 1961), nutritional, endocrine (Silberberg and Silberberg, 1941; Silberberg et al., 1961) and environmental (Ghadially, 1978; Perrin et al., 1978) influences. Morphological changes in cartilage vary among species as age advances. These changes are well described in mice (Silberberg and Silberberg, 1941; Silberberg et al., 1961, 1964), rabbits (Barnett et al., 1963; Davis et al., 1962), dogs (Lust et al., 1972; Lust and Sherman, 1973; Wiltberger and Lust, 1975) humans (Ghadially and Roy, 1969; Ghadially, 1978) and partly in swine (Grondalen, 1974a; Nakano et al., 1979a); however no electron microscopic description of swine cartilage is available.

It has been reported that degenerative joint diseases and leg weakness frequently occurs in growing pigs, and the incidence of joint lesions increases with age (Grondalen, 1974a; Perrin et al., 1978; Nakano et al., 1979a). The objective of this study is to study growth dependent changes and degenerative abnormalities in articular and epiphyseal cartilage of growing boars using light microscopy (LM) and electron microscopy (EM).

MATERIALS AND METHODS

Animals: Five groups of five newborn litter-mate crossbred (Yorkshire X Lacombe) boars were obtained from the University of Alberta herd. One boar from each of the five litter groups, was randomly selected for slaughter at 3 days and 5, 10, 20 and 30 weeks of age. Housing and management of the animals followed standard practice for Alberta described by Aherne et al. (1974).

Thickness measurements: Tissue (3 mm wide) containing visually normal articular and epiphyseal cartilage and bone were removed from each site of the right proximal femur and distal humerus as shown in Fig. 1. The measurements of articular cartilage thickness were made on sites shown in Fig. 1 using vernier calipers. The cartilage thickness was also measured in the middle of the epiphyseal cartilage in each section.

Light microscopy: Tissues used for thickness measurement were fixed in 10% neutral buffered formalin, decalcified, fixed and dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections (6 microns) were stained with Harris hematoxylin and eosin. Cellularity and vascularity were determined using scores from 0 to 3 (0: none, 1: low, 2: medium, 3: high).

Electron microscopy: Articular cartilage samples (1 mm) from the left proximal femur and distal humerus were obtained in duplicate from the sites shown in Fig. 1, and fixed immediately in 3% glutaraldehyde buffered with 0.05 M sodium cacodylate pH 7.2 for 2 h at $4^{o}C$. The tissue was postosmicated, dehydrated, and embedded in Epon 812 by routine methods. One micron thick sections were stained with toluidine blue for orientation of the block face. Ultrathin sections were stained with aqueous uranyl acetate, for 1h at room temperature and Reynolds' lead citrate (Reynolds, 1963) for 2 min. Some sections were stained either with saturated methanolic uranyl acetate or 4% phosphotungstic acid, to enhance the contrast of collagen fibres. Sections were examined with a Philips 201 electron microscope. The microscope was calibrated, using a grating containing 2160 lines per mm.

Statistical analysis: Age related changes in cartilage thickness, cellularity and vascularity were analyzed statistically using analysis of variance. Significant differences among the age means were determined using Newman Keuls' multiple range test (Steel and Torrie, 1960).

RESULTS

Light microscopy: The articular and epiphyseal cartilage were thickest in 3 day old boars and gradually decreased ($P < 0.05$) in thickness as age progressed (Table 1). The cellularity and vascularity also decreased ($P < 0.05$, Table 1) as the age of the animals increased. Eosinophilic streaks parallel to the long axis of the bone appeared in the epiphyseal cartilage from boars 10 weeks and older. Occasionally separation of the cartilage with the appearance of an empty space (likely fluid filled) occurred adjacent to the eosinophilic streaks, some of which were associated with atrophic blood vessels. Separation and space formation was also noted in some samples between the columns of chondrocytes in the epiphyseal cartilage. Such separations were seen in none of five pigs at 3 days and 5 weeks, one of five at 10 weeks, five of five at 20 weeks and one of two at 30 weeks. Articular cartilage lesions were not examined histologically in this study but were described in the distal femurs in a previous study (Nakano et al., 1979a).

The proximal femoral epiphysis of one 20 week old pig had extensive separation (Fig. 2) of the cartilage in the middle of the proliferating zone. Cartilage cells proximal to the separation were proliferating into the empty space. Primary spicule formation was continuing into the metaphysis from the cartilage on the distal side of the separation. Fracture and separation of primary spongiosa occurred in one area below intact cartilage adjacent to the separation. Occasionally focal zones of ossification failure (osteochondrosis) were present in the epiphyseal cartilage of several pigs resulting in overgrowth of cartilage (Fig. 3).

Electron microscopy: The surface of the cartilage was covered with a randomly oriented layer of fine fibrils, 6-9 nm in diameter (Fig. 4, 5). This layer increased in thickness with advancing age and also showed a loosely aggregated amorphous material between the fibrils, which formed a felt like appearance (Fig. 6). Collagen in the matrix showed morphological variations mainly in thickness and orientation of the fibrils. In 3 day and 5 week old boars, thin fibrils were arranged parallel to the surface (Fig. 7) and were approximately 30 nm in diameter. While in 10 to 30 week old boars, collagen fibrils were oriented randomly (Fig. 8) and thickened fibres of 70 nm diamter with 60 to 70 nm periodic banding were also present.

The matrix often contained evidence of necrotic cells as indicated by vacuolation, accumulation of electron dense material, myelin bodies (Fig. 9) and cellular debris (Fig. 10). Membrane bound bodies containing glycogen particles and a few strands of rough endoplasmic reticulum (RER) were commonly seen, especially in the chondrocytes from 20 and 30 week old boars (Fig. 11). The eosinophilic streaks in epiphyseal cartilage, under the EM consisted of a narrow zone of matrix which had more vesicles and fewer fibrils than adjacent cartilage. The fibrils in the streak were continuous with those in the adjacent matrix and the larger spaces surrounded by the fibrils occurred near the empty space seen histologically.

Chondrocyte morphology changed during growth of animals. Three day and 5 week old boars had elongated, spindle shaped and dividing cells in the superficial region of articular cartilage. These cells had well developed RER and several glycogen containing areas (Fig. 12). In boars 10 weeks and older, the superficial region showed round to polygonal cells containing reduced amounts of RER (Fig. 13) and other well developed organelles.

In the deeper region of articular cartilage, chondrocytes were present in pairs or clusters in 3 day and 5 week old boars. These cells showed extensive RER development and the other organelles were less well developed.

Cytoplasmic processes were small and projected into electron lucent lacunae (Fig. 14)   Chondrocyte from 10 to 30 week old boars had a reduced amount of RER and an increased number of other organelles including lysosomes, Golgi apparatus, vesicles, fibrillar structures and fibrous lamina (Ghadially et al., 1972). Glycogen particles, lipid droplets and protein-polysaccharide particles were easily resolved, and cytoplasmic processes were well developed in these cells (Fig. 15).

DISCUSSION

Light microscopy (Table 1) and the EM results reveal a decrease in cell density with advancing age. These observations are consistent with reports for swine (Nakano et al., 1979a) and human articular cartilage (Meachim and Collins, 1962; Meachim et al., 1965; Stockwell,1967). Cell death has been reported in normal cartilage(Ghadially,1978). Its rate varies among species and joints(Meachim and Collins,1962; Meachim et al., 1965). This necrosis is reflected in the form of cell remnants in the electron micrographs which include cell ghosts, membrane bound bodies and debris. Light microscopy revealed a decrease in the number of vessels with age suggesting that the thicker cartilage of young animals requires supplementary nutrition (Wolf, 1975) in addition to nutrition through synovial fluid by diffusion.

The lamina splendens of articular cartilage shown by LM (Barnett et al., 1963; Weiss and Mirow, 1972) corresponds to the fine fibrillar felt-like layer observed by EM. Our results show a gradual increase in its thickness with advancing age, which is also reported for bovine articular cartilage (Balazs et al., 1966). The lamina splendens is 0.2 to 0.3 micrometers in thickness in rabbits (Barnett et al., 1963) and dogs (Wiltberger and Lust, 1975), however no description of age related changes are available. Balazs et al. (1966) reported this layer to be adsorbed protein-polysaccharides and glycosaminoglycan of the hyaluronic acid type ("adsorbed mucin") which presumably functions as a "lubrication cushion" on the articular cartilage. Our results imply that as the animal grows older and heavier, it requires a thicker "lubrication cushion".

The collagen fibres in the matrix changed in size and distribution as the cartilage matured. Primitive thin collagen became thicker during growth showing clear evidence of sub-banding. Similar age related changes have been reported in mouse (Silberberg and Silberberg, 1941; Silberberg et al., 1961, 1964); rabbit (Barnett et al., 1963; Davis et al., 1962); dog (Lust et al., 1972; Lust and Sherman, 1973; Wiltberger and Lust 1975) and human (Ghadially and Roy, 1969) cartilage. The eosinophilic streaks in epiphyseal cartilage have been assumed to be cracks (Reiland, 1978) but there was no evidence of fracture in streaks examined by EM. This streaking is due to less dense fibres and larger vesicles and may represent weak areas in the matrix structure. Some appear to be local defects remaining from blood vessel atrophy and disappearance as the cartilage matures.

Organelle contents and distribution, in the cartilage cells, changed with age. The younger animals had well developed RER and a variable number of mitochondria, nucleoli and vesicles, indicating active protein synthesis. In the older animals, the amount of RER decreased and the number of other organelles such as Golgi apparatus, lysosomes, and vesicles increased. These observations suggest that chondrocytes are in different synthetic cycles as maturation proceeds, although it was not uncommon to find active protein synthesizing cells in mature cartilage. The cytoplasmic processes of the chondrocytes in older age groups, appeared to be involved in pinocytosis (Ghadially et al., 1972) with the release of vesicles. These vesicles contain intramatrical lipid debris and a complex distribution of protein-polysaccharide particles (Ghadially, 1978).

Epiphyseal cartilage lesions were mainly observed as separations and space formation. The incidence of these lesions increased with advancing age of animals as was reported by others (Grondalen, 1974a; Nakano et al., 1979a; Perrin et al., 1978). The severe lesions observed in the proximal femoral epiphysis of one pig represents the early lesion of epiphyseal lysis which can cause lameness if epiphyseal separation occurs. Epiphyseal lysis and slipped upper femoral epiphysis occurs in young humans especially during prepubertal rapid growth (Morscher, 1968). It may be that the traumatic forces (Grondalen, 1974b; Jussila and Paatsama, 1972) applied to the epiphyseal cartilage at the site of empty spaces near atrophic blood vessels or at eosinophilic steaks cause further separation and epiphyseal lysis. Lysis of the epiphysis occurs with osteochondrosis in swine (Reiland, 1978; Walker et al., 1966).

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Table 1. Cartilage thickness, cellularity and vascularity in the proximal femur and distal humerus

		Thickness mm				Cellularity +				Vascularity +			
		PF		DH		PF		DH		PF		DH	
Age	No. of Pigs	A	E	A	E	A	E	A	E	A	E	A	E
3 days	5	2.09^{a} (0.18)*	1.80^{a} (0.39)	1.94^{a} (0.17)	1.64^{a} (0.24)	3.0^{a} (0.0)	3.0^{a} (0.0)	3.0^{a} (0.0)	3.0^{a} (0.0)	3.0^{a} (0.0)	2.7^{a} (0.3)	3.0^{a} (0.0)	2.7^{a} (0.6)
5 weeks	5	1.78^{a} (0.42)	0.97^{b} (0.16)	2.02^{a} (0.37)	1.09^{ab} (0.45)	2.1^{b} (0.4)	2.3^{ab} (0.5)	2.3^{ab} (0.5)	2.2^{a} (0.4)	2.9^{a} (0.1)	2.2^{a} (0.4)	2.6^{a} (0.5)	2.2^{a} (0.4)
10 weeks	5	1.38^{ab} (0.41)	0.95^{b} (0.10)	1.08^{b} (0.19)	0.72^{b} (0.14)	2.0^{b} (0.0)	2.0^{b} (0.0)	2.0^{b} (0.0)	2.0^{b} (0.0)	2.2^{a} (0.4)	2.4^{a} (0.5)	2.0^{a} (0.7)	1.6^{a} (0.5)
20 weeks	5	1.08^{b} (0.17)	0.95^{b} (0.12)	0.87^{b} (0.21)	0.66^{b} (0.14)	1.0^{c} (0.4)	0.8^{c} (0.0)	1.0^{c} (0.0)	1.0^{c} (0.0)	0.6^{b} (0.5)	0.6^{b} (0.5)	0.2^{b} (0.1)	0.3^{b} (0.1)
30 weeks	3	1.10^{b} (0.04)	0.97^{b} (0.20)	0.84^{b} (0.20)	0.65^{b} (0.17)	1.0^{c} (0.0)	1.0^{c} (0.0)	1.0^{c} (0.0)	1.0^{c} (0.0)	0.5^{b} (0.5)	0.7^{b} (0.6)	0^{b} (0.0)	0.5^{b} (0.1)

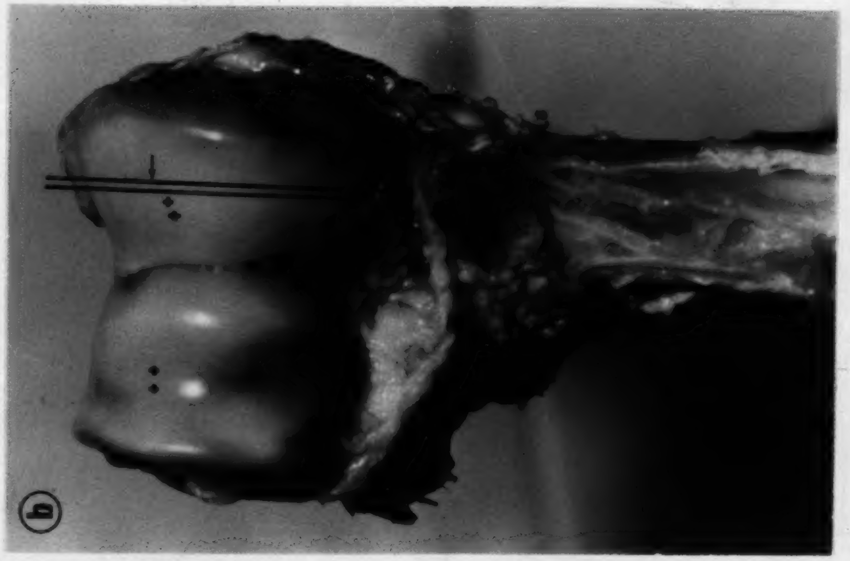
Abbreviation: PF; proximal femur: DH; distal humerus: A; articular cartilage: E; epiphyseal cartilage.

+ Scores from 0 to 3; 0:none, 1:low, 2:medium, 3:high.

* Value in parentheses indicates a standard deviation.

a-c Means in the same column with differing letters are significantly different ($P < 0.05$).

Fig. 1. Proximal femur (a) and distal humerus (b) showing sampling sites of tissues. The 3 mm thick sections were obtained by cutting the double lined area of each joint. Arrow shows the site of measurement of articular cartilage thickness. Samples for EM study were taken from the sites indicated with ' + '.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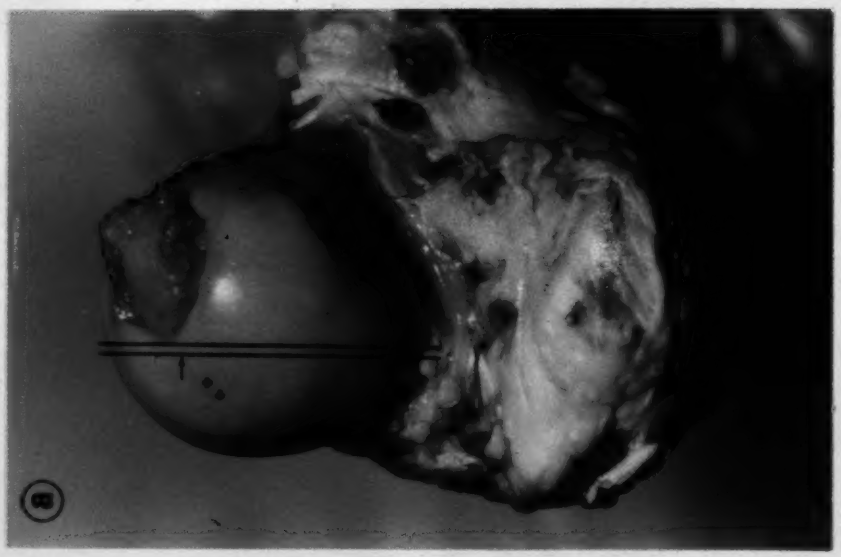
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Fig. 2. Proximal femoral epiphysis of 20 week old pig with extensive separation of the cartilage in the middle of the proliferating zone. x50.

Fig. 3. Cartilage from 10 week old animal with a zone of ossification failure in the epiphysis, resulting in overgrowth of cartilage. x50.

Fig. 4. Surface of articular cartilage from 3 day old animal showing poorly developed lamina splendens (arrow) and immature collagen. x12000.

Fig. 5 . Surface of articular cartilage from 10 week old animal showing well developed fibrous layer (arrow). Collagen fibres are oriented parallel to the surface. x15000.

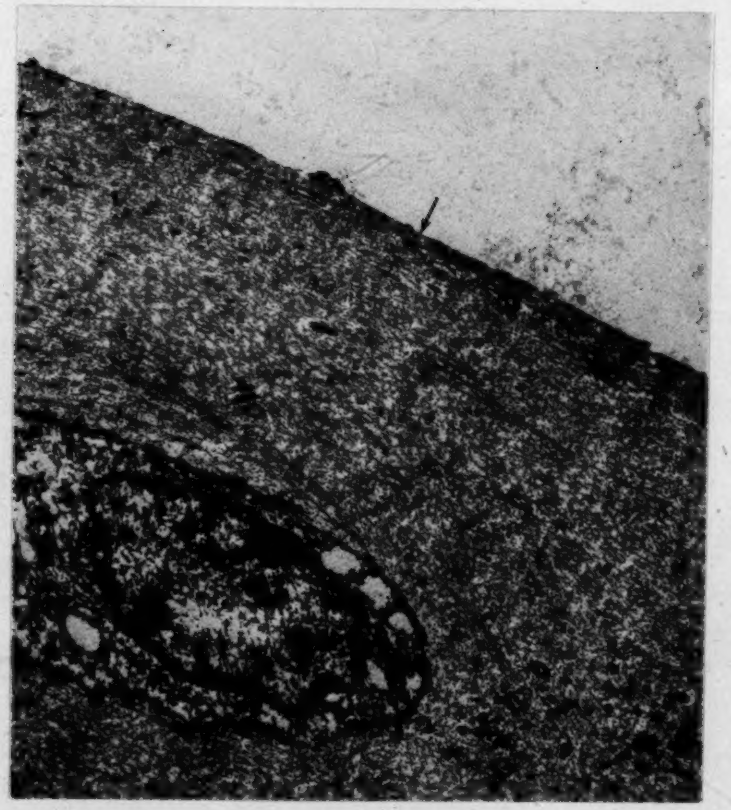


Fig. 6. Surface of articular cartilage from 30 week old animal showing thickened fibrous layer (lamina splendens). A loosely aggregated amorphous material is present between the fine fibrils, forming a felt-like appearance.

Arrow indicates direction toward the articular surface. x15600.

Fig. 7. Cartilage matrix from 5 week old animal. Thin collagen fibrils are aligned parallel to the articular surface. Arrow indicates direction toward the articular surface. x29000.

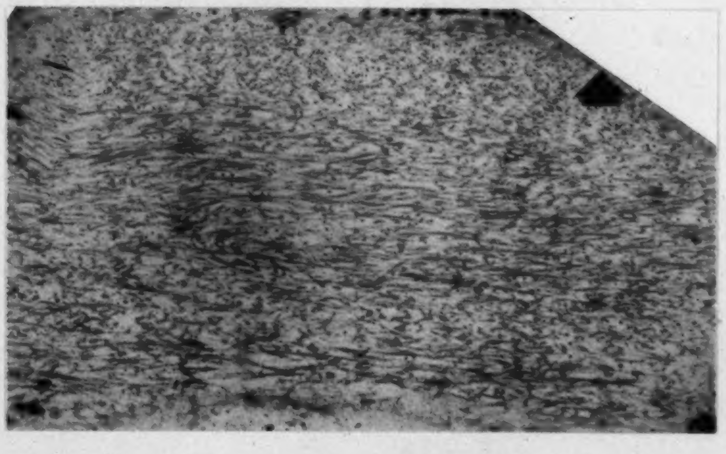
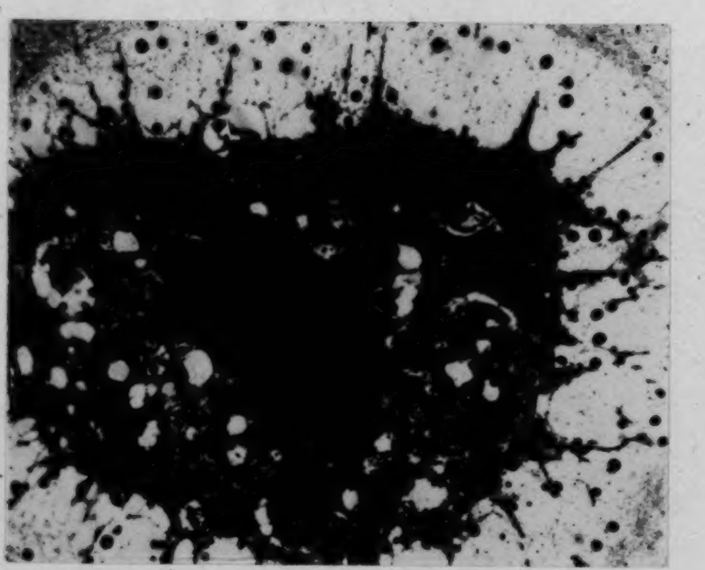


Fig. 8. Cartilage matrix from 30 week old animal showing collagen of different diameters. Thick fibres of 70 nm diameter with 60 to 70 nm periodic banding are present. x11400.

Fig. 9 . Chondrocyte from 20 week old animal containing a myelin body. x38000

Fig. 10. Cartilage matrix from 20 week old animal containing a necrotic cell. x9700.

Fig. 11. Chondrocyte from 30 week old animal showing membrane bound bodies containing glycogen and few strands of rough endoplasmic reticulum. x20800.



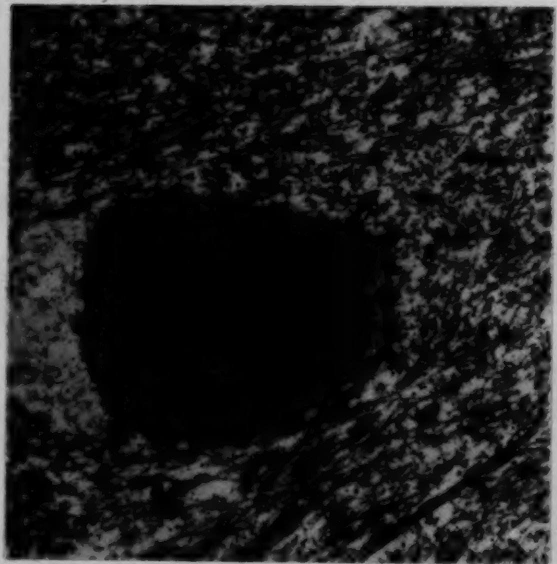
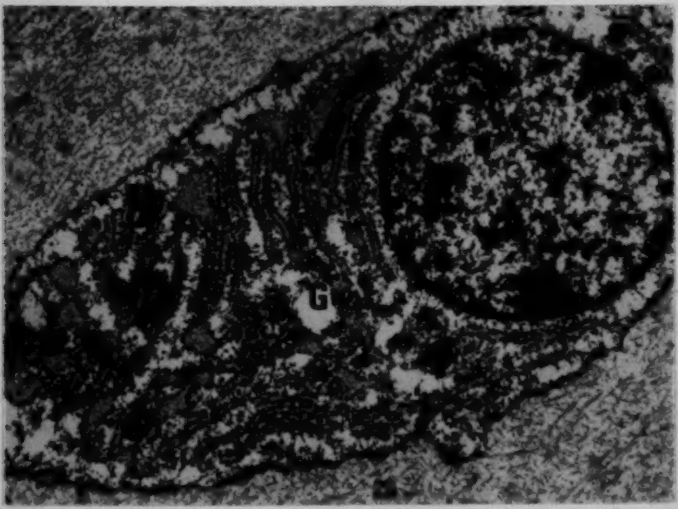


Fig. 12. Cell in the superficial zone of cartilage from 3 day old animal, showing extensive RER, few mitochondria (M) and empty glycogen containing (G) areas. x7000.

Fig. 13. Cartilage from 10 week old animal showing well developed organells and reduction in RER compared to cartilage from 3 day old animal. x10400.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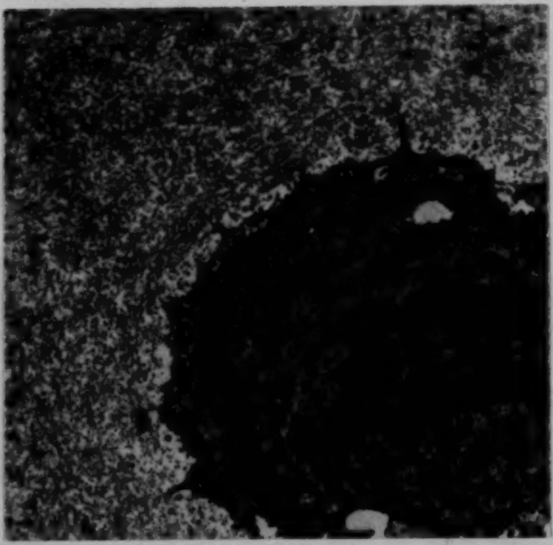

Fig. 14. Cells in the deeper region of cartilage from 3 day old animal , showing extensive RER, glycogen containing areas (G) and poorly developed foot processes (arrow). x13000.

Fig. 15. Cells in the deeper region of cartilage from 30 week old animal, showing well developed organelles and inclusions. Foot processes are also well developed and some appear in the form of vesicles (arrow). x20800.

G
G

CHAPTER 3*

CHONDROITIN SULFATE DISTRIBUTION IN STIFLE ARTICULAR CARTILAGE OF SWINE

ABSTRACT

Chondroitin sulfate (ChS) was determined histologically and chemically at seven sites from four selected areas of distal femoral articular cartilage from each of five 90 kg boars. The concentration of ChS was higher ($P < 0.05$) in the force bearing than in the non-force bearing areas of the cartilage. Site differences in cartilage thickness and collagen concentration were also observed.

INTRODUCTION

Chondroitin sulfate (ChS) is a major glycosaminoglycan component of the intercellular matrix of cartilage and contributes to maintaining mechanical strength of the tissue (Kempson, 1973). Nakano et al. (1979) have reported that ChS accounts for more than 98% of the total glycosaminoglycan content of the femoral articular cartilage in the stifle joint in the pig. The concentration of ChS is frequently used to evaluate the soundness of articular cartilage

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(Mankin et al., 1971). However studies of human articular cartilage (Bjelle, 1975) have shown that ChS concentration varies at different sites in the articular cartilage of joints. Bjelle (1975) demonstrated that ChS concentrations are higher in cartilage taking part in articulation and presumably subjected to greater force than non-articulating cartilage. No such studies have been reported for swine. Therefore, this study was undertaken to determine the concentration of ChS in the force bearing femoropatellar and femorotibial surfaces and adjacent non-force bearing surfaces of the distal femoral cartilage of swine stifle joints. This joint was selected because of the relatively high incidence of abnormal cartilage found in the articulating surface of the distal femur in swine (Grondalen, 1974; Nakano et al., 1979).

MATERIALS AND METHODS

Five Lacombe X Yorkshire boars reared under a standard confinement system, averaging 90 kg and 5.0 months of age, were slaughtered by mechanical stunning and exsanguination. None of the boars displayed signs of leg weakness. After slaughter, one femur was dissected from each animal. Transverse strips of articular cartilage (4 x 7 mm) were removed from seven different sites from four areas (I to IV) of each distal femur free of cartilage damage, as shown in Fig. 1. Each strip was divided equally along its longitudinal axis for histological and chemical analysis. Cartilage thickness was measured at the centre of each half of each strip using vernier calipers.

In this study areas I and III are termed force bearing areas while areas II and IV are termed non-force bearing areas. Areas I and III are located in the center of the femoropatellar and femorotibial articulating surfaces respectively and are considered to be subjected to considerably more force than the adjacent areas II and IV which are not normally in contact with the articulating surfaces of the patella or tibia.

Histological determination of ChS and collagen were performed using safranin O, fast green and iron-hematoxylin as described previously (Nakano et al., 1979). Safranin O is an orthochromatic dye which selectively stains glycosaminoglycan. The intensity of the color is proportional to the glycosaminoglycan content of the tissue (Rosenberg, 1971). The amount of ChS and collagen were individually evaluated using scores from 1 to 7 (1: none, 3: small amount, 5: medium amount, 7: large amount). For chemical analysis, acetone dried samples were analyzed for uronic acid and hydroxyproline as reported previously (Nakano et al., 1979). The concentrations of ChS and collagen were calculated by multiplying the uronic acid concentration by 2.86 (Kempson et al., 1970) and the hydroxyproline concentration by 7 (Simunek and Muir, 1972), respectively. Analysis of variance and Newman Keuls' multiple range test (Steel and Torrie, 1960) were used to detect significant differences between means.

RESULTS AND DISCUSSION

As shown in Table 1, cartilage thickness did not differ significantly between the lateral and the medial sites of each area.

The thickness was significantly ($P < 0.05$) greater in the force bearing (I and III) than in the non-force bearing areas (II and IV) with the exception that there was no significant difference in cartilage thickness at the medial sites of areas I and IV. Though data concerning area differences in the thickness of distal femoral cartilage of swine were not found in the literature, it is generally suggested that articular cartilage is thicker in the force bearing areas of a joint (Barnett et al., 1961). No significant difference was found in the thickness between the two force bearing areas (I and III) or between the non-force bearing areas (II and IV). There were also no significant differences in histological scores or concentrations of ChS and collagen between the lateral and medial sites of each area. Similar observations have been reported for the distal femoral force bearing and non-force bearing areas of cartilage of humans (Bjelle, 1975). The histological score for ChS was higher ($P < 0.05$), and that for collagen was lower ($P < 0.05$) in the force bearing (I and III) than in the non-force bearing (II and IV) areas with no significant differences between areas I and III or II and IV. This is consistent with the chemical observations (Table 1) except that there were no significant differences between area I and the medial site of area IV for ChS concentration or between the medial and lateral sites of area I, and the medial sites of areas III and IV for collagen concentration. Similarly Bjelle (1975), in a study of human distal femoral articular cartilage, reported higher concentrations of ChS and lower concentrations of collagen in cartilage subjected to maximal load than in the non-force bearing areas of articular cartilage.

The results obtained in this study indicate that articular cartilage is thicker and contains greater concentrations of ChS and smaller concentrations of collagen in the force bearing than in the non-force bearing areas. This reflects the significance of ChS to the force bearing and shock absorption properties of joints as suggested by Kempson (1973). There were no significant differences in cartilage thickness, histological score or concentration of ChS or collagen between the two force bearing areas, though area III in all likelihood is subjected to a greater force. The observed non-significant differences in cartilage thickness and ChS concentration between the lateral or medial sites of area I and medial site of area IV (Table 1) suggest that in area IV, the medial site is more involved in force bearing than is the lateral site.

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Table 1. Cartilage thickness and histochemical scores and concentrations of chondroitin sulfate and collagen of the distal femoral articular cartilage of swine

	Weight bearing site				Non-weight bearing site		
	I		III		II	IV	
	lateral	medial	lateral	medial		lateral	medial
Thickness (mm)	1.71(0.11)[†]^{ab}	1.84(0.15)^{ab}	1.89(0.11)^{a}	1.96(0.18)^{a}	0.64(0.10)^{c}	0.78(0.15)^{c}	1.18(0.16)^{bc}
Histochemical score							
ChS[‡]	6.4(0.4)^{a}	6.2(0.4)^{a}	6.4(0.1)^{a}	6.2(0.1)^{a}	3.4(0.1)^{b}	2.8(0.2)^{b}	3.2(0.1)^{b}
Collagen	2.8(0.2)^{a}	2.8(0.2)^{a}	3.0(0.3)^{a}	2.6(0.2)^{a}	6.0(0.3)^{b}	5.8(0.2)^{b}	5.6(0.2)^{b}
Concentration (mg/g dry weight)							
ChS	143.0(14.2)^{ab}	140.1(15.3)^{ab}	146.7(6.4)^{a}	157.3(5.9)^{a}	100.4(6.3)^{c}	89.5(13.8)^{c}	109.8(10.2)^{bc}
Collagen	522.2(11.3)^{bc}	535.5(11.6) bc	479.5(12.2)^{c}	497.7(21.6)^{bc}	603.4(21.6)^{a}	592.9(18.5)^{a}	570.5(21.6)^{ab}

[†] Value in parenthesis indicates standard error of mean.

[‡] ChS: Chondroitin sulfate.

[a-c] Means in the same horizontal row with differing letters are significantly different ($P<0.05$).

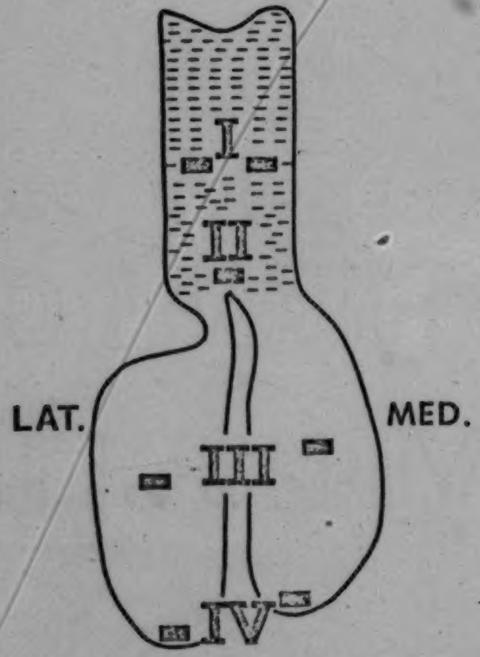

Fig. 1. Articular cartilage surface of the right distal femur showing sampling areas (I to IV) with the specific sites that were sampled shown as rectangles. The hatched area represents the trochlea while the non-hatched area represents the medial and lateral condyle.

CHAPTER 4*

EFFECTS OF FEED RESTRICTION, SEX AND DIETHYLSTILBESTROL ON THE OCCURRENCE OF JOINT LESIONS WITH SOME HISTOLOGICAL AND BIOCHEMICAL STUDIES OF THE ARTICULAR CARTILAGE OF GROWING-FINISHING SWINE

ABSTRACT

Forty crossbred pigs were reared from 28 to 90 kg liveweight to study the effects of feed intake, growth rate, sex and dietary estrogen on the incidence and severity of limb joint abnormalities. No significant treatment effects were observed. However all pigs showed some degree of osteochondrotic or osteoarthrotic lesions. Osteochondrotic joints exhibited thickening of articular cartilage and failure of endochondral ossification. The latter was associated with cell necrosis, reduced amounts of proteoglycan and collagen in the tissue, and decreased percentage of released uronic acid by tissue autolysis. Osteochondrotic joints also showed a loss of the proteoglycan or had clefts in the deeper region of the articular

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* The material in Chapter 4 of this thesis has been published in the September, 1979, issue of the Canadian Journal of Animal Science: Nakano, T., Aherne, F.X. and Thompson, J.R. 1979. Effects of feed restriction, sex and diethylstilbestrol on the occurrence of joint lesions with some histological and biochemical studies of the articular cartilage of growing-finishing swine. Can. J. Anim. Sci. 59: 491-502.

cartilage. Connective tissue was frequently found within the area of the cleft. In the cartilage beside the cleft, cell clustering and a loss of cellularity were often observed. Involvement of physical stress was suggested as an etiological factor of osteochondrosis. Osteoarthrotic joints were associated with local losses of both proteoglycan and cellularity in the articular cartilage. Cell clustering and granulation tissues were also observed in the affected regions.

INTRODUCTION

Modern swine are highly susceptible to degenerative joint abnormalities and leg weakness (Pointillart and Gueguen, 1978). Grondalen (1974a) suggests that leg weakness results from abnormal endochondral ossification (osteochondrosis) and non-infectious degeneration of articular cartilage (osteoarthrosis). Little is known about the etiology of the condition. Rapid weight gain of the pig has been suggested to be a contributing factor (Reiland, 1976). Grondalen (1974b) reported that the incidence and severity of knee and elbow joint lesions was higher in boars than in glits. This could be due in part to the fact that boars grow at a faster rate than gilts or barrows (Wong et al., 1968). Level and type of sex hormone may also be involved since in male mice, castration or administration of estrogen has been shown to reduce the degree of joint degeneration (Silberberg, 1971).

Several studies of leg weakness and joint cartilage lesions of swine have been published (Grondalen, 1974a; Perrin and Bowland, 1977; Perrin et al., 1978), but there is limited information available concerning the histological and biochemical characteristics of articular cartilage lesions of swine.

This study was designed to determine the effects of feed restriction, growth rate, sex and diethylstilbestrol (DES), a synthetic estrogen, on the incidence and severity of articular cartilage lesions in growing-finishing pigs. Morphological, histological and biochemical studies of normal and abnormal cartilage tissues were also undertaken.

MATERIALS AND METHODS

Forty Yorkshire X Lacombe pigs (24 boars, eight barrows and eight gilts) of an average initial weight of 28.4 kg were assigned on the basis of sex and initial weight to five treatment groups. All pigs were judged to be free from lameness at the start of the experiment. The pigs were housed in groups of four in concrete-floored pens (2.4 x 1.5 m) with straw used as bedding. Barn temperature was maintained at 20°C. Eight barrows, gilts and boars were fed a standard grower diet (Table 1) ad libitum. Another eight boars were individually fed the same diet at 70% of the feed intake of the ad libitum fed boars. For the remaining eight boars, DES was mixed with the diet at a level of 5.5 mg per kg diet, and the diet was fed ad libitum. Water was available ad libitum. Weight gain and feed intake were recorded weekly. The locomotory condition of each pig was evaluated before the pigs were slaughtered at 90 kg liveweight using a subjective scoring system according to the following scale:

0 = Normal
1-3 = Slightly to markedly stiff
4-5 = Slightly to markedly lame

After slaughter, all limb joints were opened and visually examined for soundness of articular cartilage. To evaluate the degree of cartilage abnormalities, subjective scores (0 to 5) were used according to the following criteria:

0 = Smooth and free of lesions
1-3 = Minor to moderate evidence of disturbed endochondral ossification and/or presence of cartilage grooves or depressions
4-5 = Moderate to severe disturbance of endochondral ossification often including cartilage clefts, and local loss or superficial fractures of articular cartilage.

All evaluations of the locomotory condition and joint cartilage were made by the first author.

Non-articulating depressions (synovial fossae) (Sisson, 1917; Doige and Horowitz, 1975) observed in certain joints (e.g. humeroradial and tibiotarsal joints) were given a score of zero.

Cartilage scores were compared only within the same type of joints and not between different joint types. Transverse strips of cartilage (2 x 5 mm at the articular surface) were separated from subchondral bone at the central area of each medial femoral condyle and their thickness measured using vernier calipers. Two measurements were made in each strip and an average was calculated. The measurements did not include thickened regions of cartilage caused by disturbed endochondral ossification.

For histological analyses, samples of articular cartilage and subchondral bone were fixed in 10% buffered formalin, pH 7.3. They were routinely processed and embedded in paraffin wax(Drury et al.,1967). Sections, 7 μ thick were cut vertical to the articular surface and stained with iron hematoxylin, fast green and safranin O (Lillie, 1965). These dyes stained nuclei, collagen and proteoglycan (a major component of cartilage matrix), respectively. All limb joints were then stored in a freezer (-30°C) until used for biochemical analysis.

Tissue autolysis was monitored on fresh thawed normal and abnormal cartilage. Normal samples (approximately 1 mm thick adjoining subchondral bone) were taken from either normal medial femoral condylar cartilage (four joints from three pigs) or non-lesion areas of osteochondrotic medial femoral condylar cartilage (five joints from five pigs). In addition, abnormal cartilage samples were obtained from the lesion areas of the medial femoral condyle of 18 osteochondrotic joints obtained from 15 pigs. In each case autolysis was monitored in triplicate on each of five pooled samples.

Tissues were prepared by finely dicing the cartilage samples into approximately 1 mm^3, and each 50 to 150 mg of tissue was incubated in 1 ml acetate buffer, pH 5.0, at 37°C for 1 h (Jibrill, 1967). Aliquots of medium were then removed and assayed for uronic acid (Bitter and Muir, 1962) to estimate the amount of proteoglycan released into the medium from the incubated tissues. Total uronic acid concentrations were estimated by pooling the released and residual uronic acid values. The latter was determined following digestion of the tissue residue with papain (McDevitt and Muir, 1976).

Autolysis was also measured on a mixture of equal amounts of normal medial femoral condylar cartilage from three pigs and abnormal cartilage resulting from a failure of endochondral ossification. The abnormal tissue, obtained from 12 osteochondrotic medial femoral condyles from eight pigs, was cut as fine as possible with a scalpel and homogenized in acetate buffer using a glass Potter-Elvehjem type homogenizer with a Teflon pestle driven by an electric drill at 1600 rpm. The amount of acetate buffer used per mg of tissue was similar to that described above for tissue incubation. Homogenization was performed in an ice bath for 2 min. Tissues were incubated using the same condition as those discussed above and the amount of uronic acid released from the mixed incubations was compared with that from incubations of normal or abnormal tissue alone.

For hydroxyproline analysis, samples were hydrolyzed in

6N HCl for 24 h at 100°C (Simuneck and Muir, 1972), and hydroxyproline concentration was determined according to the method of Stegemann and Stalder (1967). Collagen concentration was calculated by multiplying the hydroxyproline concentration by a value of 7 (Simuneck and Muir, 1972).

Analysis of variance and Newman-Keuls' multiple range test were used for detection of significant differences between treatment means (Steel and Torrie, 1960).

Joint swabs from each of 10 selected knee and elbow joints were routinely examined for bacteria and mycoplasmas at the Veterinary Services Division, Alberta Department of Agriculture. Standard protocols (Carter, 1973) were used in the search for the presence of non-fastidious bacteria and hemophilus species. A specific search for mycoplasma hyorhinis and m. hyosynoviae was made using a protocol which included three successive passages (72 h apart) in broth and on agar media in a humid atmosphere of 10% CO_2 in air at 37°C. Difco PPLO broth base without crystal violet was used, to which was added L-cysteine HCl (0.1% w/v), NAD (0.01% w/v), thallium acetate (0.1% w/v), penicillin G potassium (2000 IU/ml) and inactivated mycoplasma-free porcine serum (10% v/v). Agar was prepared using Difco PPLO agar, to which was added NAD (0.01% w/v), thallium acetate (0.05% w/v), penicillin G potassium (1000 IU/ml) and inactivated mycoplasma-free porcine serum (10% v/v).

RESULTS AND DISCUSSION

Growth Performance

Performance data are summarized in Table 2. Feed intake of boars fed the restricted level was 71% of that of the boars fed ad libitum as was intended. There was no difference in the feed intake of the barrows and the two groups of boars fed ad libitum. Feed intake of the gilts was less ($P < 0.05$) than that of boars and barrows fed ad libitum but was not significantly different from that of the boars fed the DES treated ration. These observations are consistent with reports of Newell et al. (1973) except that these authors found no significant difference in feed intake between gilts and boars. Daily gain reflected feed intake and was lowest ($P < 0.001$) for boars fed restricted levels of feed as was intended. Gilts had a slower growth rate ($P < 0.05$) than the boars and barrows fed ad libitum. It has been reported that gilts have a slower growth rate than barrows (Walstra, 1969), boars (Wong et al., 1968) and DES implanted boars (Newell et al., 1973). There was no significant difference in the average daily gain of any of the ad libitum fed boars and barrows. There were no significant treatment effects on feed to grain ratio. However Newell et al. (1973) reported a lower ($P < 0.05$) feed conversion efficiency for barrows than for boars.

Visual Observations

The average scores assigned to locomotory condition and appearance of the joint cartilage are shown in Table 3. Because of non-significant differences between right and left legs, each cartilage score was derived from an average of 16 scores from 16 joints of eight boars.

Feed restriction, growth rate, sex or DES treatment did not significantly affect the score for locomotory condition. Eight of the 40 pigs that finished the experiment were judged to be stiff or slightly lame, and were given scores for locomotory condition of 1 to 3. No pigs were assigned scores greater than 3. However every pig showed some evidence of disturbed endochondral ossification or cartilage lesions in at least one of its joints examined.

Though feed restriction significantly ($P < 0.001$) reduced growth of the swine, this did not affect ($P > 0.05$) the incidence or severity of leg joint lesions. These observations are consistent with those of Grondalen (1974b). In contrast to these results, Reiland (1976) reported that lowering growth rate of restricting level of feed intake dramatically reduced the incidence of joint lesions. Several researchers (e.g. Thurley, 1965) have suggested that rapid weight gain is the major etiological factor contributing to the incidence of joint lesions. Grondalen and Vangen (1974) studied genetically selected rapid and slow growing lines of Norwegian Landrace pigs and found a higher ($P < 0.01$) incidence and degree of joint lesions in the rapid growing than in the slow growing lines. However, it must be noted that the incidence of lesions in these lines was very high even in the slow growing line (94.6%). These data suggest that important etiological factors other than growth rate contribute to the incidence of joint abnormalities in pigs. The incidence of joint lesions has been studied in relation to a wide range of animal feeding and management practices such as levels of dietary calcium and phosphorus (Grondalen, 1974b), exercise (Perrin and Bowland, 1977) and floor types (concrete versus soil) (Perrin et al., 1978). In these studies, there were no differences among treatments, but very few of the swine were found to be free of joint lesions. Thus all of the pigs in this study developed cartilage lesions in their joints to a certain extent.

The frequency of cartilage lesions was greater in the distal humerus, distal femur, tarsus and metatarsus than in the proximal ulna, radius and femur. The higher incidence of lesions in the distal humerus than in the proximal radius is consistent with results of Perrin et al. (1978). Boars demonstrated a tendency towards greater lesion scores and frequency of joint lesions in the distal femur (Table 3). This observation is in agreement with the findings of Grondalen (1974b).

The cartilage lesions observed were designated as being either osteochondrotic or osteoarthrotic according to the criteria of Grondalen (1974a). Osteochondrotic joints showed disturbances of endochondral ossification (Fig. 1) while osteoarthrotic joints had depressions (Fig. 2) and local loss or fractures of articular cartilage (Fig. 3) without the apparent disturbance of endochondral ossification. In the present study osteochondrosis was most commonly observed in the weight bearing sites of femoral condyles with a higher incidence in the medial than in the lateral condyles. The results of Grondalen (1974a) confirm this pattern of the incidence of osteochondrosis. The bone surface beneath osteochondrotic cartilage was uneven due to a failure of endochondral ossification (Fig. 1). Osteochondrotic cartilage tended to be thickened and there was a positive correlation ($r = 0.56$, $P < 0.01$) between cartilage score and cartilage thickness. McDevitt and Muir (1976) experimentally induced joint cartilage lesions in the dog by cutting the anterior cruciate ligament of the stifle, and reported increased thickness of the proximal tibial articular cartilage due to the increased physical stress on the joint. Another morphological characteristic observed in this study was that osteochondrotic femoral condyles showed reduced steepness of the slope between the caudal summit and the intercondyloid fossa, when compared to visually normal joints. Several of the osteochondrotic joints had small clefts (1 to 3 mm in length) (Fig. 4) in the middle or deep regions of the cartilage. The location of these regions are indicated in Fig. 4. The clefts were almost parallel to the surface of subchondral bone. Similar observations of osteochondrotic clefts have been reported by Grondalen (1974a). These changes in cartilage thickness and femoral condylar surface together with the findings of McDevitt and Muir (1976) suggest the involvement of physical stress as a factor in the etiology of osteochondrosis.

Depressions of osteoarthrotic cartilage, which ranged from 5 to 25 mm^2 in area, were observed most frequently in the distal humerus (Fig. 2), but were also observed in the tarsus, metatarsus and proximal femur. Local loss or superficial fractures of cartilage ranging from 1 to 10 mm^2 in area were most frequently located in the cartilage of the semilunar notch of the ulna (Fig. 3) and in the tarsus and metatarsus.

In the present study, no pathogenic bacteria or mycoplasmas were found in any of the joints examined. This is consistent with previous studies of leg weakness in swine (Elliot and Doige, 1973; Grondalen, 1974a; Nakano et al., 1979).

Histological and Biochemical Observations

Visually normal articular cartilage was densely stained with safranin O due to the presence of abundant proteoglycan. This strong staining reaction masked the staining of collagen in the matrix. Many of the cleft free osteochondrotic cartilage samples showed a histological pattern similar to that of the superficial and middle regions of normal cartilage. However several of these samples showed diminished proteoglycan staining in the middle and deep regions. Cells in the areas of weak proteoglycan staining were often pyknotic. The degree to which the incidence of pyknotic cells is related to the reduction in proteoglycan is not clear. However since proteoglycan is involved in maintaining the strength of the cartilage matrix (Kempson, 1973), the loss of proteoglycan gives rise to weakening of the tissue, which may lead to the development of the clefts observed. The clefts frequently contained connective tissue (Fig. 4) which stained strongly with fast green with no detectable safranin O coloration, suggesting that the connective tissue consisted primarily of collagen with little proteoglycan present. Cell clustering and a local loss of both proteoglycan and cellularity were also observed in the areas around the clefts (Fig. 5). Very little information is available regarding the histology of cartilage clefts or the basis of their development.

Tissue displaying disturbed ossification (Fig. 6) showed a marked reduction in staining of both proteoglycan and nuclear material, suggesting reduced proteoglycan levels and cell necrosis. The necrotic cells were scattered individually throughout the matrix in contrast to viable cells which were arranged in columns. Similar observations in osteochondrotic tissue were obtained previously (Nakano et al., 1979). The affected tissues also contained smaller ($P < 0.05$) collagen concentrations than did the deep layer of visually normal cartilage (275.3 ± SE 49.6 versus 454.0 ± 15.8 mg/g acetone dried tissue).

Ossification requires prior enzymatic degradation of the cartilage matrix (Serafini-Fracassini and Smith, 1974; Lohmander and Hjerpe, 1975). Therefore, the observed collagen and proteoglycan loss in the affected tissues suggests that the hydrolytic process was initiated, but that the hydrolase activity was not adequate to degrade sufficient amounts of cartilage to permit normal ossification to occur. Hydrolase activity was monitored in regions of normal cartilage and abnormal cartilage that resulted from a failure of endochondral ossification. The percent release of uronic acid was approximately five times lower ($P < 0.05$) in the abnormal (5.1 ± SE 1.0%) than in the normal tissue (24.0 ± 6.3%). When the normal tissue was mixed with an abnormal tissue homogenate, the amount of uronic acid released from the normal tissue was 2.3 ± 0.2 mg uronic acid per g fresh tissue compared to 4.7 ± 0.4 mg per g for the control incubation. These results suggest (1) that enzymatic activity was lower in the abnormal than in the normal tissue due to the presence of an enzyme inhibiting factor or (2) that there was a factor in the abnormal tissue which inhibited the colorimetric reaction of uronic acid. These areas were not pursued further because of insufficient abnormal tissue available.

In the osteoarthrotic joints, cartilage depressions showed a weak staining reaction for proteoglycan only when cartilage scores were 3 or greater (Fig. 7). For tissues with slight depressions or grooves (scores 1 to 2), the extent of the proteoglycan staining reaction was similar to that of visually normal cartilage. Therefore, in this experiment, cartilage samples obtained from proximal radii and femurs showing maximum scores of 2 (Table 3) were not associated with the reduction of proteoglycan content. It is not known what causes these depressions and grooves or whether they disappear or are further aggravated with advancing age of animals. Cellularity did not change greatly with the degree of cartilage depression.

Local loss or superficial fractures of articular cartilage (Fig. 8 and 9) were accompanied by a reduced staining reaction of proteoglycan and clustering of cells in the affected tissue. These histological observations are similar to those described for the osteochondrotic tissues surrounding clefts. Some of the osteoarthrotic cartilages showed granulation tissue (Figs. 8, 9 and 10) which is associated with the healing of damaged tissue. Light microscopic observations of the granulation tissues showed high vascularity of the tissue containing macrophages, neutrophils and erythrocytes. No evidence of hemorrhage was observed. Intense collagen and very weak proteoglycan staining reactions were observed in the granulation tissues and their surrounding areas (Fig. 9). Loss of proteoglycan will result in both reduced weight bearing capacity and nutrient supply to the tissue (Sokoloff, 1969; Kempson, 1973).

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Table 1. Composition of grower diet

Ingredients, %	
Barley	31.5
Wheat	50.0
Soybean meal (47% protein)	15.0
Iodized salt	0.5
Calcium carbonate	1.0
Calcium phosphate	1.0
Vitamin-mineral premix*	1.0
Composition (calculated)	
Crude protein, %	16.0
Digestible energy, Mjoule/kg	13.4

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*The premix provided the following per kg of diet: 120 mg zinc; 10 mg copper; 48 mg manganese; 100 mg iron; 0.1 mg selenium; 7,500 IU vitamin A; 700 IU vitamin D_3; 45 IU vitamin E; 12 mg riboflavin; 40 mg niacin; 27 mg calcium pantothenate and 28 µg vitamin B_{12}.

Table 2. Average performance of pigs fed restricted levels of feed or fed ad libitum

Treatments	Boars restricted	Barrows ad lib.	Gilts ad lib.	Boars ad lib.	Boars ad lib. + DES	SE
No. of animals	8	8	8	8	8	
Initial weight, kg	28.5	29.9	27.2	28.7	27.9	2.32
Final weight, kg	90.1	90.2	90.7	90.6	90.4	0.67
Daily feed, kg	2.06a	3.00b	2.58c	2.91b	2.74bc	0.06
Daily gain, kg	0.63a	0.86b	0.79c	0.90b	0.90b	0.01
Feed:gain ratio	3.26	3.48	3.26	3.23	3.04	0.34

[a-c] Means with the same letters or no letters are not significantly ($P>0.05$) different.

Table 3. Average scores[+] for visual appraisal of walking condition and articular cartilage abnormalities in major joints.

	Boars restricted	Barrows ad lib.	Gilts ad lib.	Boars ad lib.	Boars ad lib. + DES	Range of score	Signi- ficance	SE
No. of animals	8	8	8	8	8			
Walking condition	0.25[+] (2)[§]	0.25(2)	0.25(1)	0.37(1)	0.25(2)	0-3	NS	0.234
Cartilage abnormality								
Front legs								
Distal humerus	1.06[‡] (12)[ϕ]	1.19(13)	0.94(16)	0.94(12)	0.94(12)	0-3	NS	0.176
Proximal ulnae	0.44(5)	0.50(8)	0.63(6)	0.69(7)	0.63(7)	0-5	NS	0.162
Proximal radius	0.44(6)	0.44(7)	0.44(6)	0.50(6)	0.50(5)	0-2	NS	0.155
Hind legs								
Proximal femur	0.56(6)	0.38(7)	0.44(6)	0.38(6)	0.38(5)	0-2	NS	0.142
Distal femur	2.00(14)	1.62(12)	1.12(9)	1.75(12)	1.75(13)	0-5	NS	0.268
Tarsus	1.06(14)	1.19(15)	1.25(14)	1.31(11)	1.25(12)	0-3	NS	0.284
Metatarsus	1.38(12)	1.31(12)	1.25(14)	1.25(13)	1.19(11)	0-4	NS	0.162

[+] Higher scores indicate poorer locomotory condition or an increased degree of cartilage abnormalities.
[§] Value in parenthesis indicates the number of abnormal pigs.

[‡] Average of right and left legs.
[ϕ] Value in parenthesis indicates the number of abnormal joints.
NS, not significant ($P>0.05$).

Fig.1. Surface appearance of subchondral bone of the medial femoral condyle after removal of articular cartilage.

A) Normal: Cartilage score, 0. B) Osteochondrotic: Bone surface is uneven with areas of disturbed endochondral ossification. Cartilage score, 4.

A

B

Fig.2. Depression of osteoarthrotic articular cartilage in the distal humerus. Cartilage score, 3. Arrow indicates the lesion.

Fig.3. Local loss or superficial fracture of articular cartilage in the semilunar notch of ulna (osteoarthrosis). Cartilage score, 5. Arrow indicates the lesion.

Fig.4. Section from osteochondrotic medial femoral condyle. Arrow shows the cleft of cartilage. a) Connective tissue stained with fast green but not safranin-O. b) Bone. A,B and C indicate approximate area of superficial,middle and deep regions. Scale bar = 0.65 mm.

Fig.5. Section from osteochondrotic medial femoral condyle. a) Cartilage associated with weak staining reactions for proteoglycan, loss of cells and cell clustering. b) Cleft. c) Deep region of cartilage close to subchondral bone. Scale bar = 0.12 mm.

⑤

④

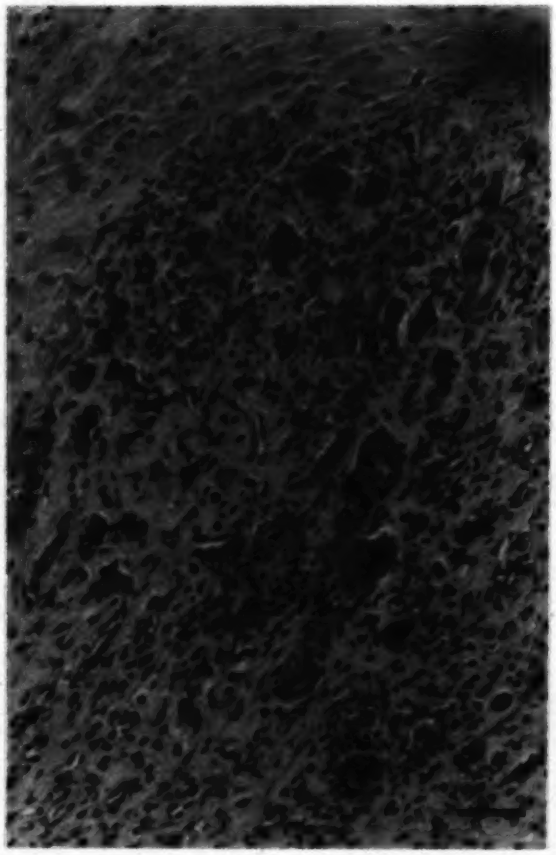
Fig.6. Section from an osteochondrotic medial femoral condyle. a) Deep region of cartilage with intense staining reactions for both proteoglycan and nuclei. b) Tissue displaying disturbed endochondral ossification with weak staining reactions for proteoglycan and nuclei. c) Ossifying area. Scale bar = 0.11 mm.

Fig.7. Section from osteoarthrotic distal humerus with cartilage score, 3. a) Normal cartilage stained intensely with safranin-O. b) The area of cartilage depression with diminished safranin-O staining. c) Bone. Scale bar = 0.60 mm.

Fig.8. Section from osteoarthrotic cartilage from the semilunar notch of ulna (Fig.3). Arrow shows the area where cartilage was lost. a) Normal cartilage. b) Cartilage associated with weak staining reaction for proteoglycan, loss of cells and cell clustering. c) Granulation tissue. d) Bone. Scale bar = 0.60 mm.

Fig.9. Section from osteoarthrotic cartilage from the semlunar notch of ulna. a) Granulation tissue with negligible safranin-O staining reaction. b) Cartilage associated with weak safranin-O staining reaction ,loss of cells and cell clustering. c) Cartilage with slightly weak safranin-O staining reaction, where nutrient supply to cells appeared to be limited due to the presence of clefts (arrow). d) Clustered cells distributed throughout the whole cartilage. e) Bone. Scale bar = 0.60 mm.

Fig.10. Section of granulation tissue from osteoarthrotic proximal ulna (Fig.9). Scale bar = 0.056 mm.



CHAPTER 5

MINERALIZATION OF NORMAL AND OSTEOCHONDROTIC BONE IN SWINE

ABSTRACT

Bone mineralization of the ulna and femur was studied using 20 market weight boars. On postmortem examination, 17 animals demonstrated osteochondrosis in the distal ulnar metaphysis and/or the distal femoral epiphysis with no apparent lesions in the distal ulnar epiphysis, distal femoral metaphysis or the diaphysis of either bone. Three animals showed no apparent abnormalities in any site of these bones. Lesion areas displaying abnormal ossification in the distal ulnar metaphyses had markedly reduced ($P < 0.05$) calcium and phosphorus concentrations, however, mineralization appeared to be normal in the apparently normal adjacent areas. Calcium and phosphorus concentrations were greater ($P < 0.05$) in the femur than in the ulna. There was no significant correlation between lesion scores and bone analysis values, between lesion scores and average daily gain, and between average daily gain and bone analysis values.

INTRODUCTION

Osteochondrosis is believed to be the most commonly occuring joint abnormality in young growing pigs (Reiland, 1978). The condition is associated with a disturbance of endochondral ossification and osteogenesis (Reiland, 1978). Grondalen (1974), Reiland (1978) and Nakano et al. (1979a, b, c) reported morphological and histological alterations of cartilage and bone in osteochondrotic swine joints. Bone mineralization in pigs with degenerative joint defects (e.g. arthritis and arthrosis deformans) has been studied (Weiss et al., 1973; Röpke, 1973; Perrin et al., 1978). These authors did not observe any significant reduction of calcium and phosphorus concentration in bones from affected animals. Little information is available regarding the mineralization of bones with signs of osteochondrosis in swine. This study was therefore undertaken to monitor calcium and phosphorus concentration in cancellous and cortical tissues from apparently normal and osteochondrotic bones of swine.

MATERIALS AND METHODS

Twenty Yorkshire x Lacombe boars, averaging 28.4 kg were housed in groups of four in concrete-floored pens (2.4 x 1.5m) with straw as bedding. These animals were fed ad libitum a standard grower diet containing 16.2% protein, 0.75% calcium and 0.65% phosphorus. Weight gain and feed intake were recorded weekly. At 90 kg body weight, boars were slaughtered by mechanical stunning and exsanguination. After slaughter, the ulna-radius and femur from both the right and left legs were removed from each animal. These bones were selected because of the high incidence of osteochondrosis in the epiphysis of the distal femur and in the metaphysis of the distal ulna (Grondalen, 1974). Each ulna and femur was longitudinally sectioned and visually examined for soundness of the articular and epiphyseal cartilage, and of the epiphyseal and metaphyseal cancellous and cortical bone. Subjective scores (0-4) were used to evaluate the severity of osteochondrosis according to the following criteria:

0 = Normal

1-3 = Slight to severely disturbed endochondral ossification and cartilage thickening.

4 = Severely disturbed endochondral ossification accompanied by apparent trabecular bone collapse and/or bone fibrosis or chondrosis.

Samples of normal and osteochondrotic cartilage and bone were examined histologically. Each sample was routinely decalcified with 20% formic acid, dehydrated and embedded in paraffin and stained with hematoxylin and eosin, and Van Gieson's stain (Drury et al., 1967).

Chemical analyses were performed on bone from the right ulnae and femurs only. Cancellous tissue samples were obtained by taking three 2mm thick transverse sections from the central area of the distal articular surface of the ulna and 2mm thick sagittal sections from the medial condylar articular surface of the femur. Each section was sprayed with a fine jet of 50°-60°C hot water (Weidman and Rogers, 1950) until the tissue appeared to be free of blood and marrow. The samples were then separated from articular and epiphyseal cartilage and cortical bone, and pooled to obtain epiphyseal and metaphyseal samples of cancellous bone. Abnormally ossified cancellous bone (Fig. 1) was separated from adjacent visually normal areas of bone for analyses. Cortical bone samples were prepared by transversely cutting the mid section of each diaphysis to obtain an approximately 1cm thick disk, which was freed from marrow and periosteum. All bone samples were then lyophilized, defatted using petroleum ether (boiling range 37.8-56.9°C) and finely ground prior to chemical analyses.

Calcium was determined by atomic absorption spectroscopy using a SP2900 atomic absorption spectrophotometer (Pye Unicam Ltd., Cambridge, England). Phosphorus was analyzed by the metavanadate method, and nitrogen by the Kjeldahl method, both as described by A.O.A.C. (1970).

Correlation coefficients were calculated between lesion scores obtained for the distal end of the ulnae and femurs, between lesion scores and average daily gain, between lesion scores and bone analysis values and between average daily gain and bone analysis values. Data were also analyzed using analyses of variance. Significant differences between means were detected by Newman-Keuls' multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

All boars showed a normal gait during the experimental period. Upon postmortem examination, three boars were free from both ulnar and femoral lesions, while all other boars showed evidence of osteochondrotic abnormalties of varying severity in either or both the distal ulnar metaphysis and distal femoral epiphysis. The lesions occured either unilaterally or bilaterally but more often bilaterally. Because of non-significant differences in lesion scores of ulnae or femurs from right and left legs, mean scores were calculated for the bones from both legs (Table 1). Lesions in the distal femoral epiphyses were associated with a disturbance of the endochondral ossification of the deeper layers of the articular cartilage. Areas of disturbed ossification did not show any apparent evidence of mineralization. The distal ulna lesions were observed as thickening of the epiphyseal plate and abnormal bone formation, which, on histological examination, was associated with tissue fibrosis (Fig. 2). Fibrotic tissues often showed macroscopic evidence of mineralization. These lesions were typical of those reported previously (Grondalen, 1974; Reiland, 1978; Nakano et al., 1979a, b, c). Seven pigs demonstrated osteochondrotic lesions (score 1) in the trochlear areas of the distal femoral epiphyses.

The correlation coefficient between femoral and ulnar lesion scores was low ($r=0.21$, $P > 0.05$). There were also low ($P > 0.05$) correlations between average daily gain and any of ulnar metaphyseal ($r=0.18$), femoral epiphyseal ($r=0.22$) and pooled lesion scores ($r=0.15$). Similarly, Perrin et al. (1978) reported a non-significant correlation between cartilage lesion scores and average daily gain in pigs.

There were no apparent abnormalities observed in cancellous bone from the distal ulnar epiphyses and the distal femoral metaphyses, and in compact bone, in any of the animals.

Analyses of calcium, phosphorus and nitrogen were conducted on visually normal areas of epiphyseal and metaphyseal cancellous bone and cortical bone from all boars. Analytical values of these bones, with lesion scores of 0, were similar to those of bones with scores of 1 or greater in both the ulna and femur. Low correlations with coefficient values ranging from -0.25 to 0.17 ($P > 0.05$) were observed between lesion scores and any of calcium, phosphorus, and nitrogen concentrations, and the ratios of calcium/phosphorus, calcium/nitrogen and phosphorus/nitrogen in the epiphyseal cancellous and cortical bone from either ulnae or femurs. The chemical composition values were therefore, pooled for each site and are shown in Table 2. The calcium and phosphorus concentrations observed in this study were within the range of values reported for whole metacarpal (Liptrap et al., 1970; Stockland and Blaylock, 1973) and humeral cortical (Perrin et al., 1978) bones.

There was no significant difference in concentrations of calcium, phosphorus and nitrogen between epiphyseal and metaphyseal cancellous bone. Calcium and phosphorus concentrations, and calcium/nitrogen and phosphorus/nitrogen ratios were greater ($P < 0.05$) and nitrogen concentrations were smaller ($P < 0.05$) in the cortical than in the cancellous bones in both ulnae and femurs. This confirms the observations of Weidman and Rogers (1950) who studied rabbit, cat and rat bones and reported a greater degree of mineralization in cortical than in cancellous bones. Calcium and phosphorus concentrations were greater ($P < 0.05$) in femoral than in ulnar cortical and cancellous bones. Nitrogen concentrations were lower ($P < 0.05$) in the femoral than in the ulnar cortical tissues, while no significant difference was observed between ulnar and femoral cancellous bone nitrogen concentrations. Calcium/nitrogen ratios were greater ($P < 0.05$) in the femur than in the ulna with the exception of the cancellous bone of the metaphyses, which showed a similar calcium/nitrogen ratio between the femur and ulna. Phosphorus/nitrogen ratios were greater in the femoral cortical than in the ulnar cortical bone, while the ratios did not significantly differ between femoral and ulnar cancellous bones.

Three tissue samples from the distal ulnar metaphysis that showed abnormal ossification were analyzed for calcium and phosphorus. Concentrations of these minerals expressed as a percentage of lyophilized-defatted tissue were 13.2±1.87 and 6.5±0.89 for calcium and phosphorus, respectively. These lower values are consistent with histological observations reflecting larger amounts of fibrotic tissue (Fig. 2). These values were approximately 40% lower ($P < 0.05$) than those in the apparently normal bone. In conclusion it is evident that a general decreased mineralization is not characteristic of bones with regions of osteochondrosis, although the lesion area itself may have less mineralization than normal tissue.

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Table 1. Mean lesion scores for distal femoral epiphyses and distal ulnar metaphyses.

Site	Number of affected pigs (and bones)	Mean Score	Range
Distal ulnar metaphysis	13 (24)	2.1	1-4
Distal femoral epiphysis	17 (30)	2.0	1-3

Table 2. Calcium, phosphorus and nitrogen concentrations in ulnae and femurs of boars.+

	Calcium(%)	Phosphorus(%)	Calcium/Phosphorus Ratio	Nitrogen(%)	Calcium/Nitrogen Ratio	Phosphorus/Nitrogen Ratio
Ulna						
Epiphyseal cancellous	23.2a	10.6a	2.18a	5.0a	4.63a	2.12a
Metaphyseal cancellous	23.8a	10.9a	2.20a	4.9a	4.82ab	2.22a
Cortical	26.4c	12.0b	2.22a	4.5b	5.90c	2.67b
Femur						
Epiphyseal cancellous	25.0b	11.6b	2.18a	5.1a	4.94b	2.27a
Metaphyseal cancellous	25.1b	11.6b	2.14a	5.0a	4.98b	2.32a
Cortical	27.3d	12.5c	2.18a	4.0c	6.85d	3.13c
Standard Error of Mean	0.20	0.11	0.02	0.09	0.05	0.07

+ Lyophilized-defatted basis.

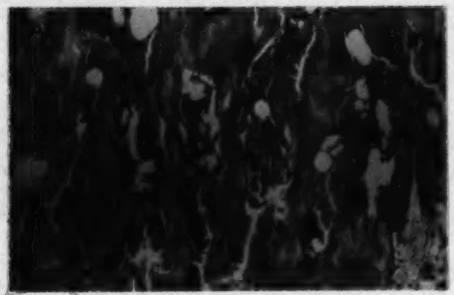
a-d Means in the same column with different letters are significantly different ($P<0.05$).

Fig.1. Longitudinal section of the distal ulna.

A: Normal. B: Osteochondrotic. Arrow shows tissue resulting from disturbed bone formation in the metaphysis.

Fig.2. Longitudinal section of cancellous bone from the distal ulnar metaphysis.

A: Normal. B: Osteochondrotic. Arrow shows immature bone.



CHAPTER 6[*]

GLYCOSAMINOGLYCAN LEVELS IN THE SERUM AND URINE OF SWINE WITH EXPERIMENTALLY INDUCED LEG WEAKNESS

ABSTRACT

Serum concentration and urinary excretion of uronic acid in papain induced cartilage degeneration of the leg joints of swine were studied. The affected joints demonstrated erosion of articular cartilage, which was associated with losses of both intercellular matrix glycosaminoglycan and cellularity. After the injection of papain, both serum concentration and urinary excretion of uronic acid were elevated ($P < 0.05$). However the increased serum or urinary levels of uronic acid were maintained for a very short time period after the injection of papain. It is suggested that serum level or urinary excretion of uronic acid is not permanently elevated in swine with osteoarthrosis, and therefore uronic acid analysis would be of limited value in detecting or verifying the presence of degenerative joint cartilage in swine.

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[*] The material in Chapter 6 of this thesis has been published in the June, 1979, issue of the Canadian Journal of Animal Sciene: Nakano, T., Aherne, F.X. and Thompson, J.R., 1979. Glycosaminoglycan levels in the serum and urine of swine with experimentally induced leg weakness. Can. J. Anim. Sci. 59: 381-384.

INTRODUCTION

Leg weakness results in an economic loss in swine production. One of the causes of leg weakness in swine is a noninflammatory degeneration of joint cartilage (osteoarthrosis) (Grondalen, 1974). Such joints showed a reduction in cartilage matrix glycosaminoglycan (GAG) (Nakano et al., 1979). The GAG lost may enter the blood circulation and appear in urine. Elevated blood concentration (Kerby, 1958) and urinary excretion (Di Ferrante, 1957) of GAG have been observed in patients with rheumatoid arthritis, and may be a useful indicator of the occurrence of the condition. However little information is available on the blood or urinary GAG levels and these relationships to osteoarthrosis. In this study, a degenerative joint condition similar to that of osteoarthrosis was experimentally induced by the intra-articular injection of papain (Bentley, 1971). Uronic acid concentrations in the serum and 24 h urinary uronic acid excretion were monitored before and after injection of papain into the knee (stifle) joint.

MATERIALS AND METHODS

Six young boars weighing 41 to 45 kg were penned individually in metabolism crates and fed a standard grower diet ad libitum. After a 3 day adaptation period, serum was obtained from 10 ml blood samples collected from each boar on days 4 and 7, by puncture of the vena cava. Twenty-four h urine collection was made for each animal on days 4, 5 and 6 with thymol (0.5 g/l urine) (Ohkawa et al., 1972) as an anti-bacterial agent. Serum and urine samples were stored at -30°C until analyzed. One h after the removal of blood on day 7, each animal was anesthetized using halothane, and 1.5 ml of 16% sterile solution of crude papain (type II, Sigma Chemical Co., St. Louis, Missouri) in 0.9% saline plus 0.5 ml of 0.12 M cystein hydrochloride as an activator was injected into the left stifle joint cavity. To the right stifle of each animal, 2.0 ml sterile saline was injected as a control. After papain injection, urine and serum samples were collected on days indicated in Table 1. Boars were exercised in a 3.88 x 1.47 m concrete floored pen on days 3 and 12 post-injection. On day 13 post-injection, boars were slaughtered. Stifle joints were opened and visually examined for the soundness of articular cartilage. Histochemical examination of the cartilage was carried out using safranin O, fast green and iron-hematoxylin staining as described previously (Nakano et al., 1979).

Serum and urine samples were prepared for GAG analysis by the methods of Emura and Mukuda (1973) and Di Ferrante and Rich (1956), respectively, and analyzed as uronic acid (Dische, 1947) with glucuronolactone as the standard. A t-test (Steel and Torrie, 1960) was used to detect significant differences between pre- and post-injection values of uronic acid.

RESULTS AND DISUCSSION

No boars showed signs of a toxic reaction to the injection of papain. All boars became lame and articular cartilage erosion was observed in the left femoral (Fig. 1) and tibial condyles. Histochemically these eroded tissues showed both a loss of chondrocytes and a very weak staining of intercellular matrix GAG (Fig. 2). The weak staining of GAG was also found frequently in non-eroded areas of articular cartilage from papain injected joints. These histochemical observations are consistent with those reported for the degenerative cartilage of humans (Bollet and Nance, 1966), dogs (Lust and Pronsky, 1972) and swine (Nakano et al., 1979). All saline injected joints were visually normal, and the cartilage showed intense staining reactions of intercellular GAG and nuclear material.

Pre-injection values of serum and urinary uronic acid (Table 1) were considerably higher than the normal values reported for humans. For example, Emura and Mukuda (1973) reported serum levels of uronic acid to be 4.1 to 6.0 μg/ml in individuals of 16 to 28 years of age. Teller et al. (1962) reported urinary excretion of uronic acid to be 7.3 ± 5.1 mg/24 h for 14 year old individuals.

The post-injection concentration of serum uronic acid (Table 1) increased and remained significantly ($P < 0.05$) higher than the pre-injection level until day 2 post-injection. On day 7 and thereafter, the concentrations were similar for pre- and post-injection period. Urinary excretion of uronic acid (Table 1) was also increased after papain injection, and was maintained at a higher ($P < 0.05$) level than that of the pre-injection period up to day 7 post-injection. On day 9 and thereafter, the uronic acid values were not significantly different from those of the pre-injection period. In the osteoarthrotic condition, the degenerative process of cartilage probably occurs at a much slower rate than that in the papain-induced condition. Therefore, one would expect a more gradual release of uronic acid from degenerating cartilage, and a rise in the serum level or urinary excretion of uronic acid may not be detectable. This is supported by unpublished data from this laboratory indicating that serum uronic acid concentration is similar between normal boars and boars which were rejected from a record of performance testing station because of leg weakness and which on post-mortem examination of the joints were seen to be osteoarthrotic.

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Table 1. Changes in serum concentration and urinary excretion of uronic acid (mean ± SD) during the experiment[+]

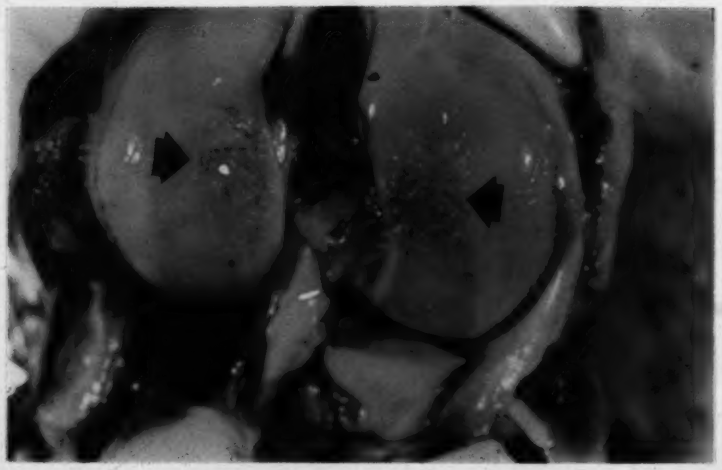
Period	Serum concentration		Urinary excretion	
	Day	µg/ml	Day	mg/24 h
Pre-injection	1-3	8.4±0.9	1-3	34.9± 7.4
Post-injection	1	11.2±0.8*	1	53.3±11.0*
	2	9.8±0.7*	4	63.8± 4.7*
	7	9.1±0.2	5	47.7±12.4*
	9	8.9±0.3	7	52.5±11.2*
	10	9.2±0.4	9	31.6± 4.6
	13	8.7±0.6	10	44.0± 6.8
			13	41.8± 6.8

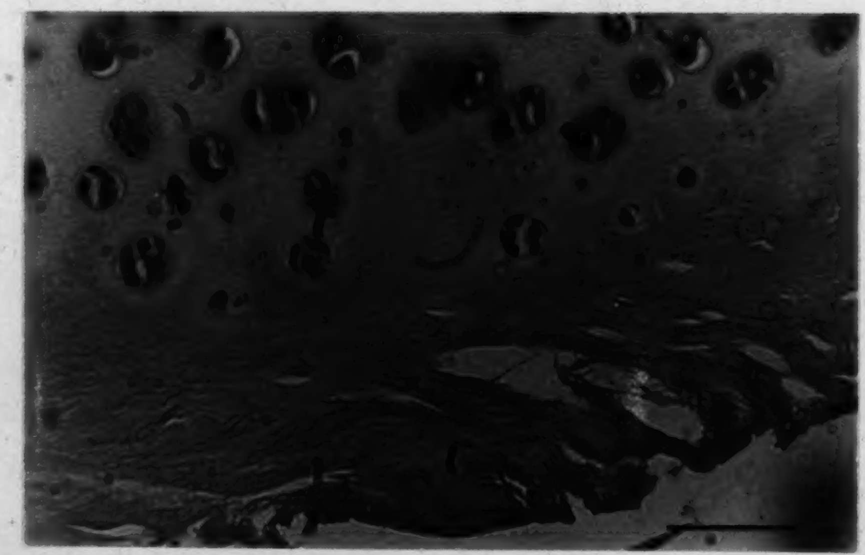
[+] Based on 15 samples for pre-injection serum, 10 for pre-injection urine, and 5 for each post-injection value.

*Significantly ($P<0.05$) higher than pre-injection level.

Fig.1. Articular cartilage erosion (arrow) in the femoral condyle from papain-injected joint.

Fig.2. Section vertical to the articular surface from eroded cartilage (Fig.1) with losses of superficial layer of cartilage, matrix GAG and cellularity. Scale bar = 0.09 mm.





CHAPTER 7

EFFECT OF HOUSING SYSTEM ON THE RECOVERY OF BOARS FROM LEG WEAKNESS

ABSTRACT

A total of 30 market weight boars, including 25 lame animals and five normal animals, were obtained from a swine performance testing station to study the effects of housing systems on the recovery of lame boars. Boars were housed in 2.0 x 1.7m concrete floor pens and allowed access to 4.0 x 23.0m indoor concrete area for 2h per day (Group I) or in outdoor sheds which opened onto 10 x 15 m dirt lots (Group II). During the seven week experimental period, there was no appreciable improvement in locomotory ability in any of the animals studied. Though gait was slightly improved in one of the boars from each group, post-mortem examination of joints indicated the presence of a considerable degree of articular cartilage lesions. The incidence and severity of joint lesions in the other 18 lame pigs were greatest ($P < 0.05$) in the proximal articular surface of ulna, distal articular surface of humerus and distal articular surface of femur. Lesion occurrence and severity were similar between boars from Groups I and II. No differences ($P > 0.05$) were observed in any of the performance characteristics among normal and lame boars which completed the experiment.

INTRODUCTION

Leg weakness of swine is a problem in which there is impairment of gait or lameness due to joint abnormalities (Reiland, 1978). Harbison (1976) reported that approximately 20% of the boars that entered performance test stations in Alberta were culled due to leg weakness. Lack of adequate exercise and hard flooring in confinement rearing systems are suggested as major contributing factors to the incidence of the condition (Elliot and Doige, 1973; Fredeen and Sather, 1978; Bereskin, 1979). Several researchers (Vaughan, 1971; McPhee and Laws, 1976) have suggested that boars can recover from leg weakness by changing their environment from confined housing to pasture or dirt lots. The present study was undertaken to examine this possibility.

MATERIALS AND METHODS

A total of 30 boars, averaging 96.1 kg and 5.4 months of age, were obtained from the record of performance (ROP) test station, Nisku, Alberta. These animals included i) 25 lame boars (11 Yorkshire, nine Landrace, two Lacombe, two Duroc and one Hampshire), and ii) five boars (three Lacombe and two Yorkshire) with normal locomotory ability (control animals). These groups of boars were rejected by the test station culling committee because of impaired locomotory ability and slow growth rate, respectively. They were carefully trucked from the test station to the University of Alberta farm. Two or three days after arrival, six lame boars were randomly selected to obtain pre-experimental information on animal gait and joint lesions. Gait was evaluated using a subjective scoring system according to the following scale.

0 = Normal
1-3 = Slight to moderate lameness
4-6 = Moderate to severe lameness
7 = No ability to stand

After gait appraisal, these animals were slaughtered by mechanical stunning and exsanguination. All limb joints were opened and visually examined for soundness of articular cartilage and subchondral bone. To evaluate the severity of joint lesions subjective scores (0-6) were used according to the following criteria:

0 = Cartilage smooth and free of lesions (normal).
1-2 = Minor to moderate degree of cartilage grooves or depressions, and/or disturbance of endochondral ossification (osteochondrosis).
3-4 = Moderate to advanced degree of cartilage grooves or depressions, and/or osteochondrosis often accompanied by local fracture and softening of cartilage.
5-6 = Severe osteochondrotic degeneration of cartilage with denuded subchondral bone, and/or cartilage lifting or separation often accompanied by collapse of subchondral bone.

All evaluations of animal gait and joint lesions were made by the senior author.

Articular cartilage and subchondral bone were also examined histologically. Samples were fixed in 10% buffered formalin, decalcified, fixed and dehydrated in ascending concentrations of ethyl alcohol, cleared in benzene and embedded in paraffin (Drury et al., 1967). Seven micron thick sections were stained with safranin-O, fast green and iron-hematoxylin (Lille, 1965), hematoxylin and eosin, and Van Gieson's stain (Drury et al., 1967).

The remaining boars were randomly divided into two treatment groups. Group I contained 13 lame and three control boars, while Group II contained six lame and two control boars. Group I boars were individually housed in 1.7 x 2.0 m pens with concrete floors and straw as bedding. After a one week adaptation period, boars were individually allowed access to a 4 x 23 m indoor area with a concrete floor so that they could exercise for 2 h a day. The temperature of the pens and exercise area was maintained at approximately 20°C. Group II boars were individually penned in large open front sheds with dirt floors and straw as bedding. Each boar housed in these sheds had continuous access to individual large (10 x 15 m) outdoor lots where it could exercise. The outdoor temperature ranged from 10 to 30°C in the daytime and from 3 to 18°C at night. Boars in both Groups were provided a standard 16% crude protein grower diet and water ad libitum.

The gait of each boar in Groups I and II was appraised at the start of the experiment and once a week thereafter, using the scoring method described previously in this paper. Weight gain and feed intake were also recorded weekly. The experiment was conducted for a 7 week period, which was considered sufficient because a previous study (McPhee and Laws, 1976) reported recovery of pigs from leg weakness within 4 weeks of arrival at the farm. At the conclusion of the experiment, boars were slaughtered and the severity of joint abnormalities was visually and histologically evaluated using the scores as described previously in this paper.

Data were analyzed using analyses of variance. Comparison of means was made using Newman-Keuls' multiple range test (Steel and Torrie, 1960). Correlation coefficients were calculated to determine the relationship between gait score and cartilage lesion score, gait score and average daily gain, and lesion score and average daily gain.

RESULTS

Gait appraisal of the pre-experimental animals showed that four boars were lame with scores 4 to 5, and two were stiff with scores 2 and 3. On postmortem examination, each animal had joint lesions with a score of 4 or greater on one of; the proximal articular surface of the ulna, the distal articular surface of the humerus or the distal articular surface of the femur.

During the adaptation period one boar from the dirt lot (Group II) with severe leg weakness (gait score 7) had a greatly reduced feed intake. This boar was withdrawn from the experiment and slaughtered so that its limb joints could be examined. Severe articular cartilage erosions (score 6) were observed in the distal articular surfaces of both femurs. All other boars were able to complete the experiment. There were no obvious disease problems with the pigs other than leg defects during the experimental period. Lame boars appeared to lie down on the floor or dirt more often than did control boars, but the difference was not recorded. In spite of the differences in environment or leg condition there were no significant ($P > 0.05$) differences in average daily gain, daily feed intake and feed/gain ratio either between lame and control boars or between boars from Groups I and II (Table 1).

Results of gait appraisal of boars at the beginning and end of the experiment are shown in Table 2. Of the 18 lame boars, nine were scored 4 to 6 (moderate to severe lameness) and four were scored 2 to 3 (slight to moderate lameness) in Group I, while two boars were scored 4 to 6 and three were scored 2 to 3 in Group II on the first appraisal (week 1). Typical examples of abnormal leg appearance are shown in Fig. 1. During the 7 week period, there was no improvement in gait of any boar that initially scored 4 to 6 in either Group I or II. Two boars (one from each group) initially rating 2 and 3 showed slight improvement in gait, with scores decreasing from 2 to 1, and 3 to 2 respectively. All control boars were scored 0 for gait in both treatment groups throughout the 7 week period.

Postmortem examination of joints indicated no significant difference in lesion frequency or severity between boars from either

Groups I and II, or between pre-experimental boars and those from Group I or II. Therefore, joint lesion scores in 13 sites from 25 lame boars were combined and are shown in Table 3. The distal articular surfaces of the humerus and femur and the proximal articular surface of the ulna were associated with the highest ($P < 0.05$) lesion scores. Lesion areas demonstrated surface irregularity, thickening, softening, fracture and separation of articular cartilage, and disturbed endochondral ossification in the humeri and the femurs. Lesions in the ulnae were associated with erosion or loss of cartilage in the central area of the semilunar notch. Lesion frequency and severity were less in the proximal ends of the humeri and femurs, distal ends of ulnae and tibiae, both proximal and distal ends of radii, tarsi and metatarsi, and scapulae. Acetabula and proximal ends of tibiae had the lowest lesion socres. Formation of repair cartilage was observed in lesion areas of joints from pre-experimental boars and Group I and II boars. An example of repair tissue resurfacing the subchondral bone is shown in Fig. 2.

Correlation coefficients between gait scores and lesion scores in individual joint sites were 0.24, 0.31 and 0.36 ($P > 0.05$) in the proximal articular surface of the ulna, distal articular surface of the humerus and distal articular surface of the femur, respectively. However, when lesion scores were pooled for these three sites the coefficient was significant ($r = 0.45$, $P < 0.05$). There was no significant correlation between either gait and lesion scores in any of the other 10 joint sites examined, with the coefficients ranging from 0.10 to 0.31, or between gait and pooled lesion scores in all joint sites examined (r=0.24).

Histological examination, revealed that both cartilage erosion and fractures were associated with a diminished safranin-O staining reaction and a stronger eosinophilic staining reaction with hematoxylin and eosin, indicating a local loss of proteoglycans. Cell clustering and a loss of cells were observed in these lesion areas. Cartilage that failed to ossify showed cell necrosis and a loss of proteoglycans. Severe failure of endochondral ossification was accompanied by trabecular fracture and fibrosis in the subchondral region. Fibrotic tissues were stained deep red by Van Gieson's acid picrofuchsin, a dye which stains collagen. These tissues were observed to be partially differentiated into cartilaginous tissues showing positive safranin-O staining. Ossification occurred in cartilaginous tissue adjacent to the subchondral bone. Areas of repair tissue contained fibrotic tissues, which were derived from the subchondral bone area (Fig. 3). To a varying extent, these fibrotic tissues were observed to be differentiated to cartilaginous tissues which showed an intensive safranin-O staining. It was of particular interest to note that tissues undergoing repair were frequently observed adjacent to an active lesion in the same joint.

Boars with gait scores of 5 and 6 had severe joint lesions (score 6), and those with gait scores of 3 and 4 had lesion scores ranging from 3 to 5. Lesions scored from 3 to 6 occurred at least on one of the proximal articular surface of the ulna, distal articular surface of the humerus or distal articular surface of the femur from each boar with a gait score of 2 or greater. The two boars that improved their gait scores by 1 had relatively severe lesions, with scores of 4 and 5 in the humeroulnar joints. Lesions from one of the boars with a gait score of 2 include grooves, local fracture and softening of cartilage on the distal articular surface of the humerus, and local erosion of the semilunar articular cartilage with denuded subchondral bone (approximately $4mm^2$) on the proximal articular surface of the ulna. The other boar with gait score 1 also had a similar degree of local erosion of its semilunar cartilage in the left leg. Lesions with scores greater than 3 were associated with an increased synovial fluid accumulation. All control boars demonstrated some evidence of joint lesions with scores of 1 to 2 in one or more joints examined. Average lesion scores ranged from 0 to 0.2 on proximal articular surfaces of ulnae, distal articular surfaces of humeri and distal articular surfaces of femurs, and from 0 to 0.3 in the other joint sites. No significant ($P > 0.05$) correlations were measured between either gait score and average daily gain ($r=0.09$) or average daily gain and lesion scores ($r=0.01$).

DISCUSSION

In spite of the differences in environment or leg condition there were no significant differences in the performance traits among the four groups of boars studied.

It has been frequently suggested that the gait of lame pigs will improve if the animals are moved from confined rearing systems to less restricting environments (e.g. McPhee and Laws, 1976). Such a possibility was examined in this study. However, among the 18 ROP rejected boars with initial gait scores of 2 to 6, none showed any appreciable improvement in gait throughout a 7 week period of either indoor exercise or housing on dirt lots. McPhee and Laws (1976) reported that 30 out of 73 performance test rejected boars recovered within 4 weeks after returning to a farm environment. Differences between these results and those of the present study may in part be due to differences in the subjective method of gait evaluation, or possible differences in the severity of leg weakness at the start of the test.

In the present study, a slight improvement of gait was observed in two of the boars. An improvement of animal gait by enforced exercise has been reported by Grondalen (1974b) and by Perrin and Bowland (1977). The evidence of joint resurfacing supports the findings of Fredeen and Sather (1978), who reported articular cartilage repair in pigs released on pasture. Further support for the idea that cartilage repair can occur is provided by studies of experimentally induced femoral condylar cartilage lesions in rabbits (Mitchell and Shepard, 1976; Cheung et al., 1978). These authors reported that drill-hole injuries were filled with cartilaginous tissue when the perforation extended into subchondral bone. In contrast, when the perforation did not reach the bone, no repair tissue was observed. These observations suggest that formation of repair tissue is dependent upon activity in the subchondral bone.

Joint lesions observed in the pre-experimental boars and those from Groups I and II were similar to those reported previously (Perrin and Bowland, 1977; Perrin et al., 1978; Nakano et al., 1979a, b) except for the osteochondrotic lesions in the femoral trochlea. This is the first report of osteochondrotic lesions in the femoral trochlea of swine, but the occurrence of similar lesions has been reported in the corresponding site of human femurs (Lindén, 1976). The histological observations of the joint lesions were consistent with our previous reports of osteochondrotic swine joints (Nakano et al., 1979a, b).

The experimental results indicate that severity of joint lesions is greatest in the distal articular surfaces of the humerus and femur and the proximal articular surface of the ulna. These observations are consistent with the finding of Grondalen (1974a, b) and Nakano et al. (1979b). The significant correlation observed between gait score and pooled joint lesion scores in the three sites of elbow and knee joint suggest that lesions in these sites are associated to a certain extent with the impaired gait of the pigs studied.

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Table 1. Average performance of boars during the experimental period

Treatment	(Group I) Concrete floor		(Group II) Dirt lot	
Gait	Lame	Normal	Lame	Normal
No. of boars	13	3	5	2
Initial Wt. (kg)	98.0(3.0)[+]	97.3(2.3)	105.7(2.1)	97.5(1.5)
Final Wt. (kg)	137.2(3.4)	133.1(2.5)	143.9(1.0)	134.3(1.0)
Daily feed (kg)	3.04(0.07)	3.07(0.10)	2.87(0.09)	2.91(0.14)
Daily gain (kg)	0.80(0.05)	0.73(0.03)	0.78(0.04)	0.75(0.05)
Feed: gain ratio	3.80(0.19)	4.21(0.15)	3.68(0.19)	3.88(0.18)

[+] Value in parentheses indicates standard error of mean.

Table 2. Number of boars in each gait category after the 1st and 7th week of the experiment

Locomotory ability		Normal	Lame						Unable to stand
Gait score		0	1	2	3	4	5	6	7
GROUP I	Week 1	3	0	2	2	4	2	3	0
	Week 7	3	0	3	1	4	2	3	0
GROUP II	Week 1	2	0	2	1	1	0	1	0
	Week 7	2	1	1	1	1	0	1	0

Table 3. Mean joint lesion scores

Site	Score[+]
Distal humerus	3.96 (1 - 6)[‡]a
Distal femur	3.70 (2 - 6)ab
Proximal ulna	3.26 (1 - 6)b
Proximal radius	1.80 (0 - 4)c
Distal tibia	1.68 (0 - 3)cd
Tarsus	1.60 (0 - 4)cd
Proximal humerus	1.26 (0 - 3)cd
Proximal femur	1.25 (0 - 4)cde
Metatarsus	1.20 (0 - 3)cde
Distal ulna-radius	1.08 (0 - 2)cde
Scapula	0.94 (0 - 3)de
Proximal tibia	0.68 (0 - 2)e
Acetabulum	0.66 (0 - 2)e

[+] Scores for right and left legs were pooled as the values were not significantly ($P>0.05$) different, thus each value was derived from an average of 50 scores from 25 lame boars.

[‡] Value in parentheses indicates score range.

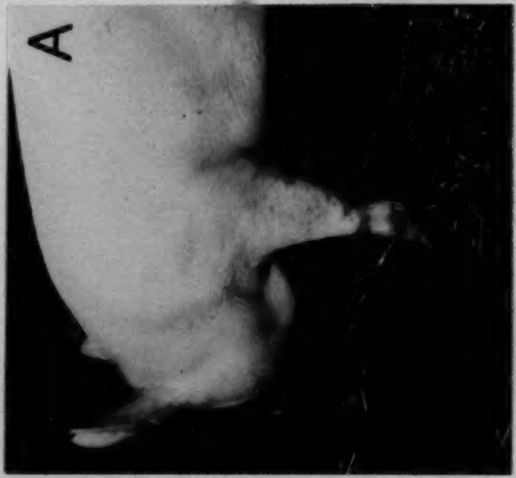
a-e Means with different letters are significantly ($P<0.05$) different.

Fig.1. Abnormal leg appearance.

A: Normal Yorkshire boar.

B: Lacombe boar showing ' up on toe '.

C: Yorkshire boar with cross legged gait.


A

B

Fig.2. Severe osteochondrotic humeral condyle from a 6 month old Yorkshire boar.

a) Detached articular cartilage on the medial condyle (arrow).

b) The area beneath the detached articular cartilage on the same condyle showing repair tissue (arrow) resurfacing the subchondral bone.

b

Fig.3. Histological section of an osteochondrotic medial humeral condyle from a 6 month old Yorkshire boar, showing repair tissue containing fibrotic tissue

a) Articular surface. b) The area of intensive safranin-O staining. c) Subchondral bone. Section was stained with safranin-O, fast green and iron-hematoxylin. Scale bar = 0.23 mm.

a

GENERAL DISCUSSION

Leg weakness in pigs is a serious problem associated with modern production methods. In this thesis, a series of studies were undertaken to investigate the etiological factors of joint abnormalities and leg weakness in swine.

In general, gross, microscopic and biochemical changes observed in swine articular and epiphyseal cartilage during growth were consistent with those reported in the cartilage from other species including humans and dogs (Chapters 1 and 2).

Osteochondrosis, which was manifested as a non-infectious disturbance of endochondral ossification, was the major joint abnormality in the pigs studied. Severe osteochondrotic lesions were associated with lameness (leg weakness) of the animals. The frequency of lesions was similar among boars, barrows and gilts (Chapter 4). Articular cartilage of the elbow and knee joints as well as of the distal epiphyseal plate of the ulna were the sites most commonly affected. The frequency tended to be greater in the medial than in the lateral condyles of both the humerus and femur. Gross and histological examination of early developmental stages of the lesion revealed failure of ossification in the deep calcifying region of the cartilage. In the more advanced cases, fracture of the cartilage and/or subchondral bone occurred. Fractured cartilage frequently appeared to be softer than normal cartilage (Chapters 1,4,5 and 7).

The study of knee joints from boars showed that the incidence and severity of osteochondrosis increased with increasing age and body weight of the animals (Chapter 1). A visual estimate of the slope of the weight-bearing surface from caudal summit to intercondyloid fossa revealed a reduced steepness of the slope in affected joints (Chapters 1 and 2). The steepness decreased with increasing severity of the lesions (Chapter 1). It was suggested from these observations that mechanical stress due to increasing body weight contributes to the incidence of lesions, although it may not be a primary factor.

Osteochondrotic cartilage tended to be thicker than normal cartilage. There was a significant correlation ($r = 0.56$, $p < 0.01$) between lesion severity and thickness of the medial femoral condylar cartilage (Chapter 4). Weight stress was considered to be involved in the induction of tissue thickening. Similarly, evidence of cartilage thickening due to mechanical stress has previously been reported by others (Chapter 4). If articular cartilage thickness is increased, diffusion of nutrients from the synovial fluid, the major means of nutrient transport in this cartilage, may become less efficient. This speculation may be reflected by the frequent histological observation of the presence of pyknotic cells and a loss of matrix proteoglycans in the middle or deep regions of the thickened femoral condylar cartilage (Chapter 4). Proteoglycans in the extracellular matrix are considered to be important in controling nutrient transport to and waste removal from the chondrocyte. Proteoglycans also contribute to the compressive strength of cartilage (Chapter 3). Thus a loss of proteoglycans in the cartilage will result in impairment of the physiological functions of cells, and therefore in tissue weakening.

Although not pursued in this study, analysis of mechanical properties of cartilage may provide an insight into the development of osteochondrosis. When cartilage bears weight, thickened cartilage may be distorted to a greater extent than normal cartilage, provided that the chemical and physical properties of the cartilage are otherwise relatively similar.

Histological observations revealed chondrocyte necrosis and a loss of proteoglycans in the cartilage resulting from disturbed ossification. Microscopically, these necrotic cells were scattered individually throughout the matrix with little evidence of their columnar arrangement observed for adjacent normally maturing chondrocytes (Chapters 1 and 4). One could speculate from the above observation that these cells died before they matured. Chondrocyte maturation in the deep (calcifying) region of the cartilage is the normal process leading to endochodral ossification. Cell ghosts were retained in the matrix of most tissues that failed to ossify, which was in contrast to the matrix morphology of fractured cartilage showing disappearence of both cells and proteoglycans (Chapters 1 and 4). From these observations, one may speculate that circulatory disturbances in, or adjacent to, the affected tissue may be involved in the retention of cell debris. Unfortunately, blood circulation in the joint was not monitored in these studies. The reduced proteoglycan and collagen concentrations in the osteochondrotic tissue (Chapter 4) may be related to a reduced weight bearing capacity of this tissue. This suggestion is supported by the observation that the tissue appeared to be very soft when tested with a blunt probe.

Histological and chemical analyses demonstrated a loss of proteoglycans in the fractured articular cartilage, which may contribute to tissue weakening as discussed earlier. However, the pathogenesis of tissue weakening remains unclear. The author's recent studies of proteoglycan structure in normal and osteochondrotic cartilage of swine indicated : 1) proteoglycans are more readily extracted with 4M-guanidinium chloride, and the extracted proteoglycans are less aggregated in the severe osteochondrotic than in the normal articular cartilage, and 2) chain length of chondroitin sulfate is reduced in the cartilage with severe osteochondrosis. These structural changes of proteoglycans appear to be due in part to hydrolytic enzyme activity in the cartilage and/or synovial fluid. Involvement of lysosomal hydrolases in proteoglycan degradation in cartilage has been frequently reported. Abnormalities in chondroitin sulfate metabolism in osteochondrotic cartilage are implicated by the results presented in Chapter 1 which showed higher proportions of 6-sulfated disaccharide and lower proportions of 4-sulfated disaccharide in severe osteochondrotic than in visually normal cartilage. Limited information is available on proteoglycan metabolism in swine cartilage. Research on this area in relation to endocrine and genetic factors, and to factors related to feeding and management practices (e.g. amount of exercise, floor types etc.) appears to be important to determine the etiological factors of joint abnormalities.

Severe osteochondrotic joints demonstrated subchondral bone damage in the area adjacent to the cartilage that failed to ossify (Chapters 1,5 and 7). Tissue softening in the affected cartilage, noted previously in this discussion, might have resulted in an increased physical stress to the trabeculae. The observed fibrosis and chondrosis in the subchondral regions (Chapters 1,5 and 7) appear to be a healing responce to the trabecular damage. A study of mineralization of ulnae and femurs from boars demonstrated that calcium and phosphorus concentrations are similar between normal and osteochondrotic bones (Chapter 5), suggesting that calcium and phosphorus metabolism was normal in these animals.

In conclusion, osteochondrosis was the major joint lesion in the young growing pigs studied. The condition appears to be due to a disturbance of chondrocyte maturation. At present, we do not know if the primary cause of the lesion is due to metabolic or mechanical factors.